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ABSTRACT

This document provides biology experiments designed for students who have completed a first year biology course. This self contained laboratory booklet contains four sections. In section 1, "Instrumentation in the Study of Cells," discussion sections and suggestions for teacher demonstrations are provided. It also includes some basic materials which should have been introduced in the first year biology course, but does so in greater depth. Section 2, "Diversity of Life and Reproduction," is primarily descriptive and covers phyla normally not explored in a first year course. Section 3, "Animal Growth and Behavior," contains primarily experimental laboratories ranging from a traditional descriptive dissection of a cat to the latest in biological inquiry, the creation of hybridomas and monoclonal antibodies. Section 4, "Plant Growth Relationships," contains laboratories which are experimental in design and comprised of both classic experiments in the field, such as Darwin's phototropism experiment, and relatively new experiments, such as those involving autoradiography. (ZWH)

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ED 374 018

Action Biology

Advanced Placement for the Second Year

Mary Pitt Davis

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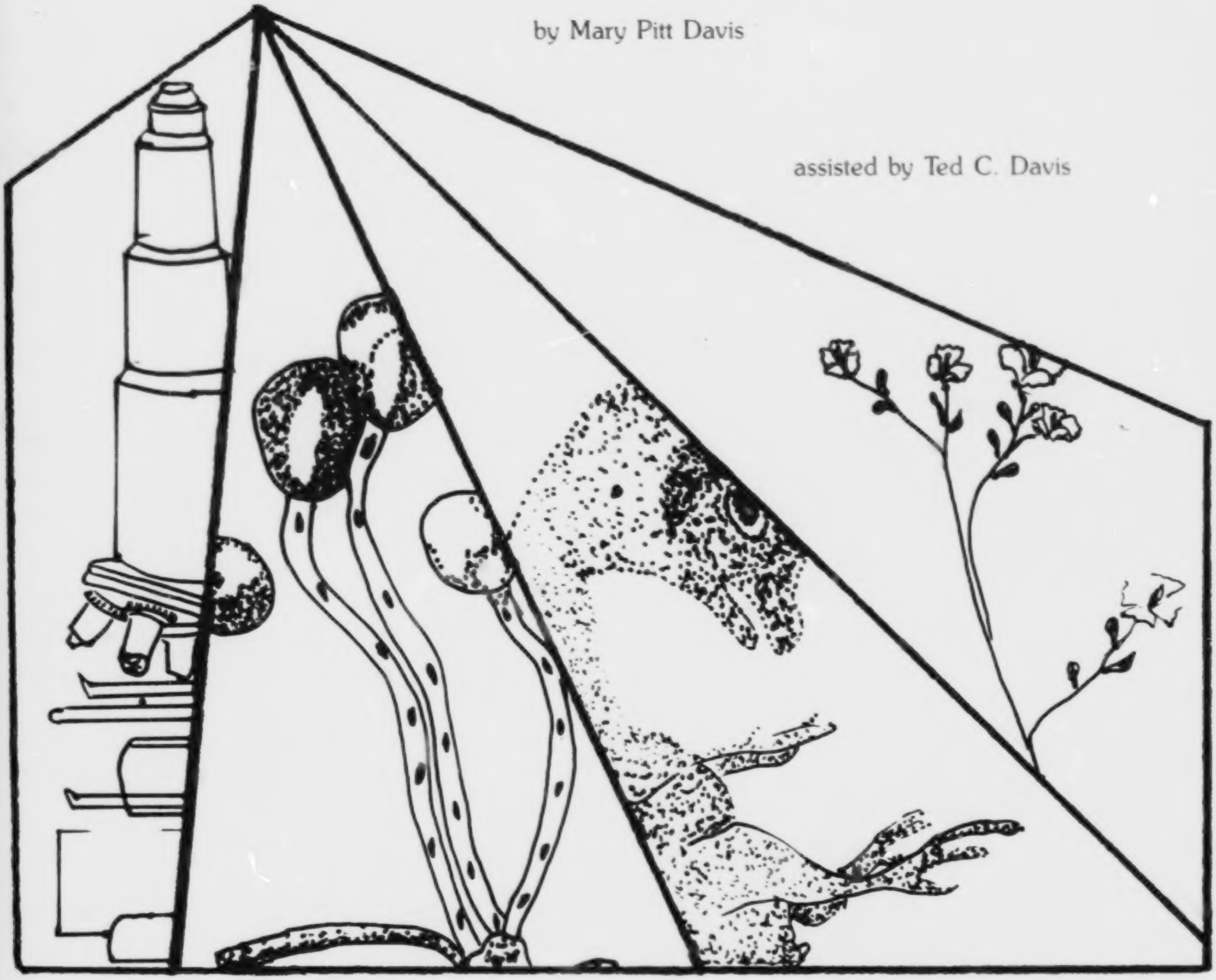
Action Biology

Advanced Placement for the Second Year

a self-contained laboratory manual — no separate teacher's manuals or student workbooks required

by Mary Pitt Davis



assisted by Ted C. Davis

Illustrated by Judy Swanson

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by
Mary Pitt Davis

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I dedicate this book to my father, Harold M. Pitt,
whose life is my example.

Preface

Over the years I have been blessed with a husband and three children, three college degrees, and sixteen years teaching experience in Washington, Japan, California, Massachusetts, Maryland, and Turkey. Following my husband around the world, first when he was a graduate student and later as a federal government employee, has led to a variety of experiences. Of these, several stand out.

Dr. H. Weston Blaser, now deceased, my advisor and professor at the University of Washington in Seattle, greatly influenced my approach to intellectual growth and pedagogical method. Former students of his will recognize some of his methods within this laboratory manual.

Sr. Patricia Culhane, also now deceased, while headmistress of the International School of the Sacred Heart in Tokyo, Japan, encouraged creative curriculum development and urged development of an advanced biology program which would combine the use of American textbooks with the needs of those who had experienced the British form system. It was a joy to teach biology to students who already had completed chemistry, but it also was a constant challenge to conduct laboratories appropriate for older students with a chemistry background. While at Sacred Heart I developed the nucleus for many of the laboratories included in this manual. Sr. Culhane asked, when we left Tokyo, that I continue to develop the laboratories and my ideas on advanced biology for high school students. Sr. Culhane, I have done so.

The Howard County Maryland Public Schools, particularly Barbara Jewett, science department head at Oakland Mills High School, and Paul M. Keyser, science director for the Howard County Maryland Public Schools, provided the freedom and the classes which allowed me to test and perfect the laboratories in this manual. This effort reflects the strivings for excellence of the Howard County school system.

Dr. Roland S. Nardone and Rene Filipowski of the Catholic University of America in Washington, D.C. gave the training I needed to prepare the laboratory on heterokaryons for high school students. Rene also developed the procedural steps and materials lists for the heterokaryon laboratory.

Dr. Yahya Laleli of Duzen Laboratories in Ankara, Turkey provided the technical information required to complete the laboratory on gel electrophoresis techniques.

Mrs. Judy Swanson of the George C. Marshall School in Ankara, Turkey prepared all illustrations for this manual.

Mr. Clark S. Carlile, the publisher, deserves a special thanks for assisting me in this project. I shudder to think how this book might have appeared without his constant good judgement and sure hand.

My husband, Ted C. Davis, most especially deserves credit for all that he has done. Quite simply, this book might not have been written without him. Not only did he pressure me to approach a publisher in the first place, but he spent most evenings, weekends, and holidays over the course of a year reviewing every word and critiquing every entry. It gives me great pleasure to acknowledge a debt to my husband and to thank him for all his forbearance with the computer and me.

Finally, I would like to thank my students over the years. After all, this book is for them.

Note to Users

Biology, the study of life, is a vast and complex subject, and one in which knowledge is rapidly expanding. The needs of both instructors and students, who face a wide range of possible topics, were considered during the selection of laboratories for this manual. Those areas of biology not adequately introduced in first year high school courses received more focus. Upon completion of this manual, in conjunction with an appropriate text, the student will have finished the equivalent of a university first year biology course and should be prepared to sit for the biology advanced placement examination administered by the Education Testing Service. *Action Biology, Advanced Placement for the Second Year,* like *Action Biology for the First Year,* is the result of years of classroom use and testing with high school biology students.

Design Assumptions

Action Biology, Advanced Placement for the Second Year, is designed for the student who has completed first year biology. It also is designed for the student who has completed chemistry, or who, at the minimum, is enrolled concurrently in chemistry. Although basic biology and chemistry are essential for effective utilization of this manual, physics is optional. Those discussions and laboratories which require some knowledge of physics are supplied with necessary background information.

This manual is designed to be used in conjunction with any of the university introductory biology textbooks currently on the market. *Action Biology, Advanced Placement for the Second Year,* is divided into one hour laboratory periods, but those schools with a two hour period, or even the three or four hour college laboratory, can use the manual simply by covering several one hour blocks during a single laboratory session.

The number of laboratories included in this manual exceeds those required during the course of a typical academic year by even the most strongly laboratory oriented instructor. With extra laboratories available, each class can wind its own path through the material, selecting those for which the school has appropriate equipment, facilities, growing conditions, and preferences. Choices may be made from the four general groupings of laboratories and discussions, which range from the sub-cellular to the social, from the descriptive to the experimental. The four parts are: 1. Instrumentation in the Study of the Cell, 2. Diversity of Life and Reproduction, 3. Animal Growth and Behavior, and 4. Plant Growth Relationships.

The manual also is self-contained. It includes background information specific to the instrument or procedure, full discussion of laboratory preparations, detailed procedural steps, places for students to enter data and discuss issues, suggestions for further projects, review quizzes, answer keys, etc. All in one, the teacher and student no longer must juggle several different manuals.

Student Objectives

Students are urged to review the objectives listed at the beginning of each laboratory or discussion and to determine, after completion of the unit, if they have accomplished those objectives. Also, students should review the terminology listed at the conclusion of each unit to verify that they know the listed words. Definitions for all words listed in the terminology sections are found in the glossary, Appendix A of this manual. Students may have to review portions of first year biology, or find access to a scientific dictionary, because it was assumed, when preparing the terminology lists and glossary, that terms introduced during the first year were learned at that time. Every subject has its own technical terminology, and biology is no exception. Students will have to know the terms which belong to the subject.

Time Line

Action, Biology, Advanced Placement for the Second Year, is designed to be used in fifty to sixty minute segments. These segments may be spaced daily, as in the most typical school schedule, or by unit, as in two to four hour blocks of time during the week. Even the discussion units, included because texts generally do not cover those topics in sufficient depth for effective laboratory utilization, are designed around the one hour time block.

Each of the four parts of the manual require from twenty two to twenty six hours of laboratory time to complete. The shortest laboratories are in the first part, Instrumentation in the Study of Cells. As students become accustomed to working with one another and handling increasingly complex experiments, the laboratories tend to become longer and require greater student organization and team effort. By part three, Animal Growth and Behavior, students are required not only to interact with classmates but also to observe the larger school community. In part four, Plant Growth Relationships, there is an experiment on the flowering response in plants which requires the entire class to coordinate and schedule a group effort. This experiment is part of a much larger laboratory which intermittently runs for one month.

Instrumentation in the Study of Cells

The four parts of the manual are designd to minimize the problems of a typical school year, in that part one, Instrumentation in the Study of Cells, begins with discussion sections and suggestions for teacher demonstrations. It then covers some basic materials which should have been introduced during the first year biology course, but it does so in greater depth and in ways designed to acquaint the student with equipment and instrumentation techniques used in more advanced biological studies. It is believed that every student should acquire facility with scientific instruments. The instructor is urged to make every effort to expose the students to as many of the laboratories in part one as is economically and physically possible. If complete coverage is not feasible, a thorough reading of the discussion units would still be useful. Over the years a stock of instruments should be acquired by your school.

Diversity of Life and Reproduction

The laboratories in part two, Diversity of Life and Reproduction, are primarily descriptive, and cover phyla normally not explored in a first year course. Students will acquire a fuller understanding of life processes and the great diversity of life forms, as well as master new laboratory techniques. Again, the discussions in the laboratories are designed to cover material pertinent to the topics covered within a laboratory but not normally discussed in standard textbooks. You may observe in this section that I have rearranged some classifications within the plant kingdom in ways which are taxonomically more accurate in terms of evolutionary relationships, and also more logical when the plant kingdom is viewed in its entirety. Write care of the publisher and tell me what you think.

Animal Growth and Behavior

Part three, Animal Growth and Behavior, contains primarily experimental laboratories, from a traditional descriptive dissection of a cat, to the latest in biological inquiry, the creation of hybridomas and monoclonal antibodies. This part of the manual takes the student from the cellular level to vertebrate behavior. Students have the opportunity to consider ethological issues and patterns of social organization.

Again, the manual is designed to minimize the problems of a typical school year, in that the last laboratory in part three is a paper and pencil exercise available to the instructor who must begin year-end closing of the laboratory at this point. Assuming that all instruments required for part one were available, and further assuming that your school schedules five hours per week in science, you might be able to cover in their entirety only parts one, two and three of this manual. If, because of equipment shortages or personal preferences, you have omitted some of the laboratories in parts one, two, and three, you likely will have time to continue on to part four, Plant Growth Relationships. The same will be true if your school schedules a double period advanced placement class, or a larger block of laboratory hours.

You also could study part four after part two, Diversity of Life and Reproduction, for the sake of continuity, or you may wish to study it at the end of the school year, if the climate in your locality favors such an approach.

Plant Growth Relationships

Part four, Plant Growth Relationships, contains laboratories which are experimental in design and comprised of both classic experiments in the field, such as Darwin's phototropism experiment, and relatively new experiments, such as those involving autoradiography. Part four introduces students to new biological techniques and equipment, such as the proper use of radioactive substances in plant physiology studies. If time does not permit complete coverage of this part, a reading of discussion sections would still be useful.

Student Groups

Materials and equipment lists provided with each laboratory are for a student group of two individuals. The teacher accordingly will have to multiply supply requirements by the number of student groups in each class. It is extremely important that every student receive hands-on experience during the laboratories. The shy or reluctant student, or even the most outgoing, learns far less in a large group laboratory arrangement.

Laboratory Preparation

All instructions for laboratory set-up are included in this manual. There is no separate teacher's guide. Your students, who may well pursue medical or scientific careers, should learn what is involved in the organization and preparation of laboratories, and aid in their set-up, if at all possible. Instructors will need to practice thorough, advanced planning, in order to stock all necessary supplies. This planning is best accomplished the previous spring. Appendix B at the end of the manual lists those suppliers with which the author is familiar.

The Laboratory

In *Action Biology, Advanced Placement for the Second Year*, procedural steps are detailed and space is interspersed for students to enter data, observations, and conclusions. Responsible students can conduct the laboratories without direct instructor supervision, once all materials and supplies are made available. In fact, some of the descriptive laboratories are self-programmed in their approach.

Testing

Student data, observations, and conclusions can be checked through periodic collection of manuals. The reviews placed on tear-out sheets at the end of each discussion and laboratory can be used either as a student self-testing and review device, or as a formal quiz. If the latter option is selected, the quiz may be removed in advance. An answer key to both laboratory questions and unit reviews for the entire manual is supplied as Appendix C on tear-out sheets. Instructors may either leave these sheets in the manual, for students to perform their own diagnostics, or tear them out prior to the beginning of the school year.

Further Research

Suggestions are offered throughout the text regarding further research possibilities. Selected additional reading resources are listed at the conclusion of each unit. These are highly selective, with an eye to availability of the material in a typical community, as well as to its comprehensibility by the students. These resources serve as a starting point for more in-depth research. Instructors should consider having students perform a major research project during the year, providing them with the resources, materials, community contact, and other general support required. The student should have intellectually matured to the point that a formal research report would be meaningful.

These laboratories and discussions are offered with the hope that they prove as useful to your biology program as they have been to mine. If you have any suggestions, please let me know care of the publisher.

Mary Pitt Davis

Action Biology

Advanced Placement for the Second Year

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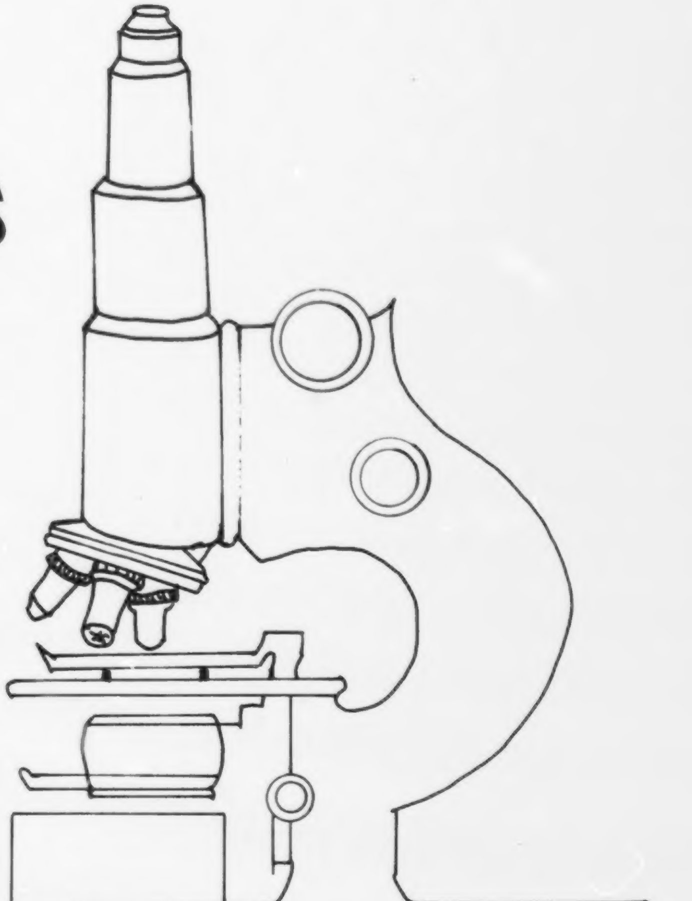
Instrumentation In The Study of Cells



1. Discussion

Development of Coacervates

When you have completed this discussion you should be able to:

1. Explain the Oparin theory of spontaneous generation and define coacervates.
2. Outline Stanley Miller's experiments creating amino acids from gases.
3. Detail Sidney Fox's experiments with amino acid chains and define microspheres.
4. Discuss the scientific issues surrounding abiotic origins.

Questions about Living Matter

What distinguishes living from nonliving matter? What is an organism as contrasted to an aggregation of organic molcules? Some aggregates can accumulate substances from their surroundings, catalyze them internally for energy, and then duplicate themselves. To be able both to metabolize and to reproduce defines the living. But whence came this capability? And how did living organisms, the first organisms, arise?

It is known that humans give birth to humans, frogs produce frogs, and wheat yields wheat. This is the commonsensical principle of biogenesis, or life from life. Louis Pasteur of France provided a series of classic experiments in 1862 which demonstrated that life cannot appear spontaneously from nonliving materials, such as frogs from mud, or maggots from meat. Life yields life. And yet, from where came the first life which produced all later generations?

Oparin Theory

A modern theory of spontaneous generation was advanced in 1924 by the Russian biochemist, A. I. Oparin. It was not until 1936, with the publication and translation into English of Oparin's new book, The Origin of Life on Earth, that his ideas began to impact upon the world's scientific community. Many could not accept the Russian's hypotheses, but his concepts could not be dismissed out of hand. Years later Oparin was awarded the Nobel Prize for his theories on the chemical conditions which might have yielded the first living organisms. Oparin's work has spawned a continuing series of experiments about the origin of life on earth.

Oparin hypothesized a series of chemical reactions leading to the first metabolizing and reproducing organisms. The first step would be the formation of small organic molecules, such as amino acids, organic bases, and monosaccharides. Next would be the formation of structural polymers, such as proteins, fats, and carbohydrates, from the small organic molecules. The final step would be the integration of the intermediate structural polymers into organisms.

Oparin suggested that the atmosphere of the early earth contained those elements and molecules which could combine to form the first building blocks of life. These would have included hydrogen, methane, ammonia, water, etc. The question then arose as to how thermodynamically stable compounds could react to form simple amino acids, organic bases, and monosaccharides. Some external source of energy would have had to act upon the mixture. Possibilities included solar radiation, lightning, vulcanism, radioactive decay, and cosmic rays.

Miller Experiment

A young graduate student working at the University of Chicago, Stanley Miller, conducted a series of experiments in 1953 which demonstrated that the building blocks of life could be synthesized abiotically. Now teaching at the University of California, Berkeley, Miller received the Nobel Prize in 1958.

Miller set up an airtight apparatus in which four gases, hydrogen (H_2), ammonia (NH_3), methane (CH_4), and water vapor (H_2O), were circulated past electrical discharges from tungsten electrodes. He kept the gases circulating continuously for one week and then analyzed the contents of the apparatus. He found that a large variety of organic compounds had been synthesized, including those essential for living systems.

This experiment by Miller, which provided the first solid evidence that Oparin might have been on the right track, marked a turning point in the scientific approach to the question of how life began. To verify the experiment, Miller repeated it several times, as well as altering key variables in the original experiment. He circulated the four gases for a week without any energy input, and no organic molecules were formed. He sterilized the apparatus at 130°C for 18 hours to ensure the absence of any living organism which could contaminate the results; this experiment yielded the same results as the original experiment. The synthesis of organic molecules had been achieved in the absence of any living organism, a synthesis of the biotic from the abiotic. Miller also varied the energy source by using ultraviolet radiation; the experiment still worked. Precursor organic compounds had been formed by supplying energy to a primordial soup of gases.

In the years since 1953 scientists have extended Miller's results by synthesizing a great number of organic compounds through reducing mixtures of gases in which the initial carbon source has been methane. Most of the organic molecules thus formed are perishable, because they react slowly with molecular oxygen. They also are broken down by organisms of decay. However, the prebiotic atmosphere of early earth contained little free oxygen, and it certainly contained no living organisms. Theoretically, then, precursor molecules were able to accumulate in both the atmosphere and in the primordial waters in which they were deposited over an extended period. Large accumulations are not possible today, because of atmospheric oxygen and decay organisms, even though scientists continue to detect organic molecules after thunderstorms and volcanic eruptions, and upon the surface of dust particles high in the atmosphere.

Polymer Formation

It has been suggested that while early organic compounds were dispersed on dust particles in our turbulent, primitive atmosphere, thereby displaying a large, reactive surface area, the chemical reactions giving rise to the first intermediate sized molecules took place. The ancient oceans of the world, or, as George Wald of Harvard University put it, "the primitive soup," was another possible reducing area for the production of beginning and intermediate sized organic molecules. Even though each such polymerization reaction, in the absence of an appropriate enzyme, is a remote statistical possibility, on a monumental time scale enough unlikely events might have occurred to produce the necessary polymers which precede life. George Wald has said, "given so much time, the 'impossible' becomes possible, the possible probable, and the probable virtually certain."

Fox Experiment

Certain concentration mechanisms, which could have facilitated the polymerization of organic molecules, might have been provided by the emergence of land. Sidney W. Fox of the University of Miami demonstrated that if a nearly dry mixture of amino acids is heated, polypeptide molecules, or amino acid chains, are synthesized, especially if phosphates are present. This situation could have appeared in such natural situations as the evaporative shrinking of lagoons, ponds, or puddles in which amino acids had been deposited. The sun would have both concentrated the acids and provided the energy required for polymerization reactions. The adsorption of amino acid compounds on surfaces such as clay particles might also have provided the large surface area needed for such reactions to proceed.

It is worth noting that in both the Fox and Miller experiments polymers were often obtained first, and amino acids only later, after hydrolysis of the polymers. These experiments suggest that abiotic syntheses may differ fundamentally from biochemical syntheses, in that polymers may be formed first, rather than the predicted amino acids. If correct, these findings would significantly shorten the sequence theorized by Oparin.

Coacervates

The final step in the Oparin sequence would be the integration of the polymers into organisms. Of course, this final step is a quantum leap which would have to result in an organic aggregate that could both metabolize and reproduce. The integration would have to be orderly, so that the organism could acquire and use energy in a systematic fashion. Such an integration would result in an endergonic, or energy using system. It would have to be able to accumulate substances from its surroundings and catalyze them in chemical reactions. Phrased differently, it would have to be able to metabolize. And it would have to be able to duplicate itself over the generations.

What mechanism might have yielded this final leap into life? One possibility, and the mechanism demonstrated by Oparin, is a large cluster of organic compounds surrounded by a water membrane which he called coacervates. He was able to form coacervates from polymer solutions under appropriate conditions of temperature, ionic composition, and pH. Each such coacervate is a cluster of macromolecules surrounded by a shell of water in which the individual water molecules are rigidly oriented relative to the colloidal particles within. There is a definite demarcation between the coacervate droplet and the liquid in which it floats. The water shell functions as a membrane around the droplet.

Coacervate droplets have a marked tendency to adsorb and incorporate various substances from the surrounding solution, sometimes to the point of removing substances from the medium. Coacervate droplets also often form definite internal structures, the molecules within the coacervate becoming arranged in an orderly manner. As more and more materials are incorporated into the droplet, a membrane consisting of surface active substances may form just inside the water shell. Thus, the permeability of the boundary of the coacervate becomes even more selective than before. Scientists have sometimes mistaken them for bacteria and tried to classify them! They do exhibit many of the properties of living organisms.

Microspheres

A different mechanism has been suggested by Stanley Fox. He believes that coacervate droplets formed in a special way may have been the true precursors of living things. He demonstrated that droplets which form spontaneously when hot aqueous solutions of polypeptides are cooled exhibit more of the characteristics of living things. Fox called these droplets proteinoid microspheres, rather than coacervate droplets. The microspheres formed in this manner exhibit many of the properties of living cells, such as reacting to the tonicity of the solution they are placed in (shrinking in a hypertonic solution and swelling in a hypotonic one), forming a double layered outer boundary, and having an internal movement similar to cytoplasmic streaming. Microspheres can move in a pattern, contain ATP, grow in size, increase in complexity, bud in a manner similar to yeasts, and aggregate in clusters in a pattern resembling many types of bacteria.

Naked Gene Theory

A different theory about the final, integrative phase of the emergence of living organisms is called the "naked gene" theory. Some think it likely that life could have evolved merely from self replicating amino acids, or "naked genes". These "naked genes" would develop only the most cursory of protective membranes when conditions became unfavorable to their metabolic and reproductive activities. Thus it is with viruses, simple creatures with simple DNA or RNA cores and simple protein coats.

Remaining Questions

Each of the above theories, or variants on them, are subject to serious questions and require much additional work. For instance, Oparin's coacervate droplets tend to disintegrate with changes of temperature and other conditions in the solution holding the droplets. Fox's microspheres, although maintained in the laboratory, have not been demonstrated to be viable outside a controlled environment. However, evidence from outside these laboratory experiments suggests that they point in the right direction.

The most universal metabolic processes, the ones which are probably the most ancient, are similar to the means whereby Oparin's coacervate droplets and Fox's microspheres maintain themselves. For instance, cellular respiration, or a close analog, was seen in the first prebiotic systems. They used ATP as an immediate source of energy. This is a heterotrophic mechanism, which is the metabolic process most likely to have been used by the first organisms. And it is the metabolic process of coacervates and microspheres.

Assuming that Oparin's coacervate droplets point toward an explanation of the origin of life, many further questions must be answered. For example, how did autotrophs develop from heterotrophs? Which came first, DNA or RNA? At what point in time did aerobic respiration evolve over fermentation? How did the use of water as a H+ donor appear? How did cellular organelles develop? Are mitochondria and chloroplasts really symbiotic bacteria and algae? How did sexual reproduction develop in eukaryotes, making more genetic variation possible? Many additional questions about the origin of life remain. The door is wide open to the curious and creative student.

Coacervate Demonstrations

Some properties of coacervates may be explored by the following.

Coacervates: Imitation of Movement Mechanism.

An overhead projector easily shows this demonstration to an entire class. Place the lid of a glass petri dish on an overhead projector and turn it on. Place a small amount of mercury on the lid, and then place two or three drops of potassium dichromate and two or three drops of dilute nitric acid on the lid. When the mercury comes in contact with the potassium dichromate and dilute nitric acid, the mercury will imitate amoebic movement, much as coacervate droplets achieve.

Miller's Spark Discharge Apparatus

Students may wish to duplicate Stanley Miller's apparatus for the creation of amino acids:

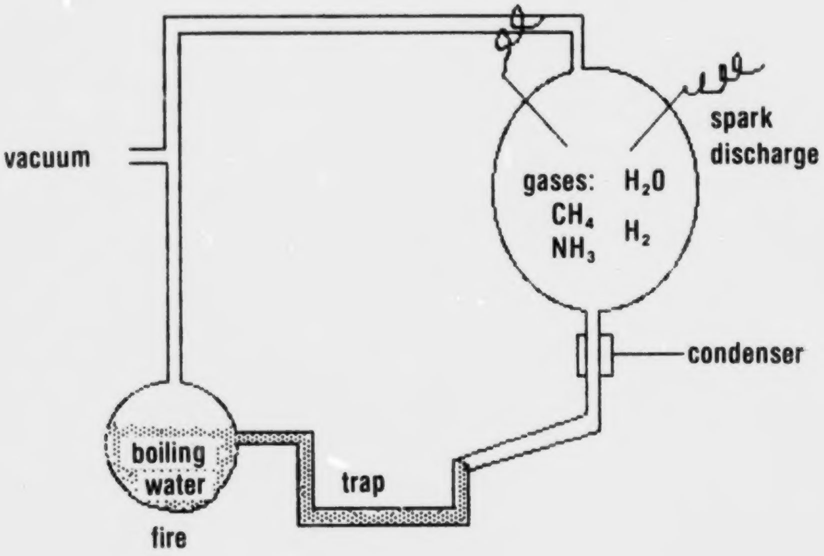


Figure 1

The apparatus can be set up and allowed to run continuously for several weeks. When students wish to check on amino acid content, they may employ some of the techniques of instrumentation they will have learned in the first unit of this manual: either the laboratory on amino acid chromatography or gel electrophoresis is appropriate.

Plotted below are some concentration changes which occurred during Stanley Miller's experiment. Will your results be similar?

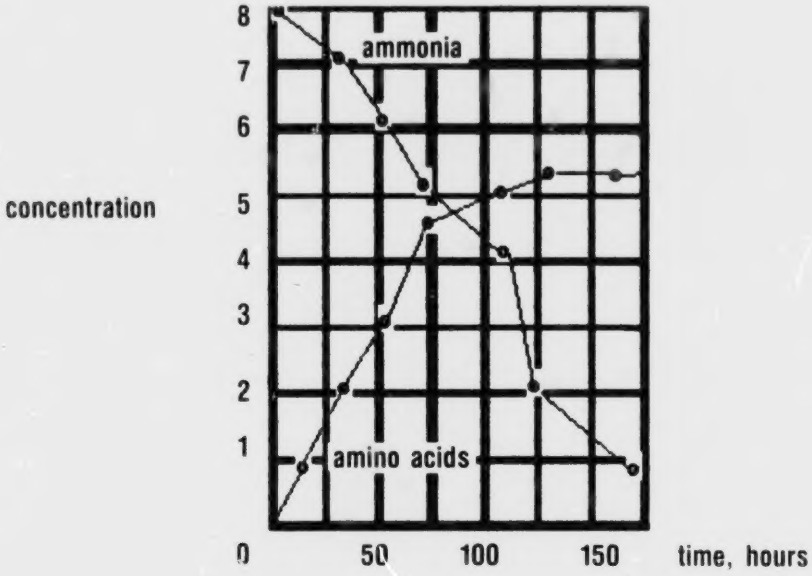


Figure 2

Water Membranes

Students may wish to consider the tensile strength of water droplets on waxed paper. What forces keep water droplets together? How is bonding of the droplets' membranes accomplished?

Student also may wish to discuss the making of air bubbles on microscope slides. What gives the air bubble in a water solution such a heavily walled shell, or membrane? Is this what is also seen in water droplets on waxed paper, but magnified?

Resources

Dickerson, Richard E. "Chemical Evolution and the Origin of Life," *Scientific American*, September 1978 (offprint #1401).

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Oparin, A. I. *Genesis and Evolutionary Development of Life*. Academis Press: New York, 1968.

Schopf, J. W. "The Evolution of the Earliest Cells," *Scientific American*, September 1978 (offprint #1402).

Wald, George, "The Origin of Life," *Scientific American*, August 1954.

Wilson, Allan C. "The Molecular Basis of Evolution," *Scientific American*, October 1985.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

- abiotic
- biogenesis
- biotic
- coacervate
- microsphere
- "naked gene"
- spontaneous generation

Review

1. Development of Coacervates

Name ______________________________

Date ______________________________

True or false

______ 1. The basis for the current theory of the origin of life was first stated by A. I. Oparin.

______ 2. Proteinoid microspheres tend to aggregate in clusters resembling those formed by many bacteria.

______ 3. The early atmosphere on earth was almost certainly an oxidizing atmosphere.

______ 4. Coacervate droplets have a marked tendency to adsorb and incorporate various substances from the surrounding medium.

______ 5. Pasteur proved the theory of biogenesis.

Multiple choice

______ 6. Who is credited with finally disproving the theory of spontaneous generation?

a) Redi
b) Spallanzoni
c) Miller
d) Pasteur

______ 7. The first high energy molecule to evolve was probably

a) ADP
b) ATP
c) CO_2
d) GMP

______ 8. Must be present in the atmosphere before aerobic respiration is possible

a) oxygen
b) nitrogen
c) ATP
d) CO_2

______ 9. Stanley Miller demonstrated that amino acids could be synthesized abiotically by using four gases. They were

a) hydrogen, ammonia, methane, and water vapor;
b) hydrogen, methane, water vapor, and carbon dioxide;
c) hydrogen, water vapor, carbon dioxide, and fluorocarbons;
d) water vapor, carbon dioxide, hydrocarbons, and methane.

______ 10. When proteinoids, or polypeptide strands, are cooled, they spontaneously form

a) coacervates
b) organelles
c) microspheres
d) cells

Essay

1. Explain how modern ideas of spontaneous generation differ from those of early biologists.

2. State Oparin's hypothesis and explain how Miller's experiment supports it.

3. Describe coacervate droplets and proteinoid microspheres and indicate ways in which they resemble living cells.

4. What are the basic requirements for life?

5. Another hypothesis for the origin of life on earth suggests that life did not originate here, but was brought by extraterrestrial objects such as meteors. The earth is constantly bombarded by meteors. Analysis indicates that some meteors contain hydrocarbons characteristic of living systems. Comment upon this hypothesis.

Answers Found: p 1 - #1, 5 & 6; p 2 - #3, 8, & 9; p 3 - #2, 4 & 10; p 7 - #7; Essay 1: entire; Essay 2: p 1 & 2; Essay 3: p 3; Essay 4: p 1; Essay 5: thought.

2. Discussion

Chromatography

When you have completed this discussion you should be able to:

1. List chromatographic types, techniques, and procedures.
2. Discuss research applications of chromatographic methods.

Background

In 1903 Mikhail Tswett, a Russian botanist, reported using a new technique for separating pigments into their component parts. He placed a solution of leaf pigments on top of a column of calcium carbonate particles and passed pure solvent down the column. The component parts of the leaf pigments were "developed" as the solvent separated them, and they were adsorbed onto the calcium carbonate particles at different levels in the column. Since these materials were colored, Tswett named this new separation process "chromatography."

The chromatographic technique today is used to separate colorless materials as well. Therefore, the best definition of chromatography is that it is a technique to separate compounds by the percolation of fluid through a body of porous, rigid material. It does not matter what the physical-chemical processes are that yield the separation. They are all considered to be chromatographic processes.

Applications

Practically all disciplines of science use chromatography. It has contributed much of our knowledge of the lanthanide and actinide elements in the periodic table of elements. It has contributed to the bulk of our knowledge of amino acids, nucleotides, and nucleoproteins. It is widely used for the microanalysis of fission products in atomic reactors. It is used for the detection of additives in foods, drugs, dyes, pesticides, perfumes, vitamins, antibiotics, and hormones. It is important in medicine for the detection of normal and abnormal constituents of blood, urine, saliva, and body tissues. It has helped scientists to trace metabolic pathways in the body and to understand various genetic defects.

Technique

In chromatography the substances to be separated are dissolved in a liquid (if it is a liquid) or evaporated in a gas (if it is a gas). This fluid enters the "moving phase" of the system in which it is passed through the interstices of a porous material. The absorbing or adsorbing material may be a finely divided solid, gel, or liquid. Separation occurs when the substances to be separated react differently over time with the solvent and the collection material. Once the solvent has passed through an apparatus, and the constituent compounds of the original complex material have differentially separated, they are in the "stationary phase" and analysis of the different compounds can begin.

Liquid Chromatography: a procedure in which solvent and solute are liquid.

Column Chromatography

It was the method used by Mikhail Tswett to separate leaf pigments. Columns are filled with particulate matter and the substance to be analyzed is dissolved in a solvent and placed at the top of the column. Pure solvent is poured over the substance and the entire solution then passes down the column. This is called elution.

When two or more solvents are used, the procedure is called gradient elution. A column procedure might proceed as follows:

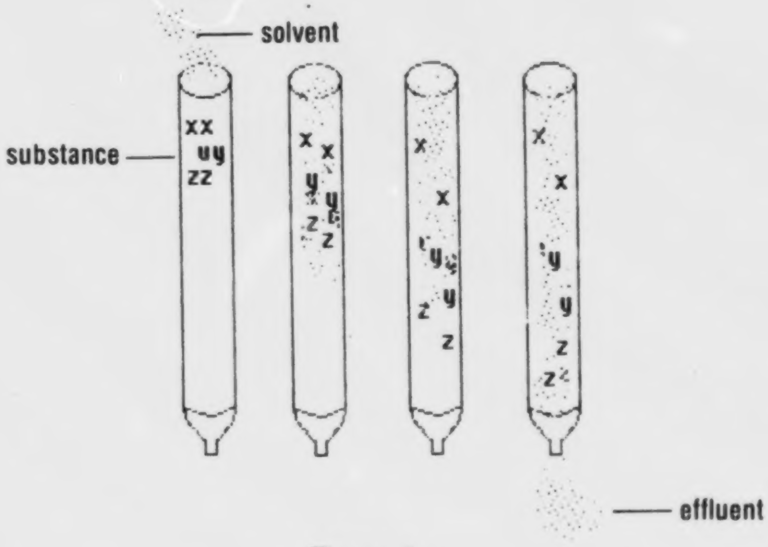


Figure 1

Remember that chromatography is merely a mechanical separation process. Analysis of the yield requires other procedures and instruments, such as measurement of optical activity with the photometer or light refractability with the spectrophotometer.

Applications

The most common type of chromatography used in protein analysis is column chromatography. The first protein structure ever to be completely analyzed was insulin. In 1953 Frederick Sanger of Cambridge University in England determined amino acid sequences in polypeptide chains of insulin by column chromatography. He used a large glass tube filled with plastic resin beads that were electrically charged. He poured insulin down the tube, and the various amino acids in the sample moved down the column at different rates, depending on their size and electrical attraction. He then washed the column with a solvent, and those amino acids which travelled fastest got washed out, or eluted, first. Sanger changed the vessels collecting the effluent frequently, so that fractions of the effluent were collected in sequence. Each vessel then contained only one, or a very few types of molecules, from the original insulin. This is called the fractionation of insulin. From these samples Sanger could determine the types of amino acids composing insulin. Through analysis of the terminal endings of the amino acids collected, and by further use of column chromatography techniques, Sanger also determined the sequence in which amino acids are positioned on the insulin protein molecule.

The subsequent discovery of bacterial restriction enzymes, which snip DNA selectively at points surrounded by certain patterns of nucleotides, permitted extension of the Sanger technique. Using the procedures and logic developed for his insulin experiments in conjunction with restriction enzyme techniques, Sanger in 1977 analyzed the entire genetic material of bacteriophage ØX174. Many DNA sequences have now been analyzed, and this procedure has provided much of the current information available on gene structure.

Paper Chromatography

The stationary material is a sheet of absorbing paper. In paper chromatography a piece of paper is spotted with the substance to be analyzed at a given distance from the bottom of the paper. This is called the base line. The paper is then hung in a chamber or jar, and the very bottom of the paper is placed in contact with a solvent. Slowly the solvent climbs the paper in a candlewicking effect, taking the substance to be analyzed and separating it into its various components by depositing them at different heights up the paper. If the solvent only ascends the paper, it is called one dimensional chromatography. The apparatus might look like this:

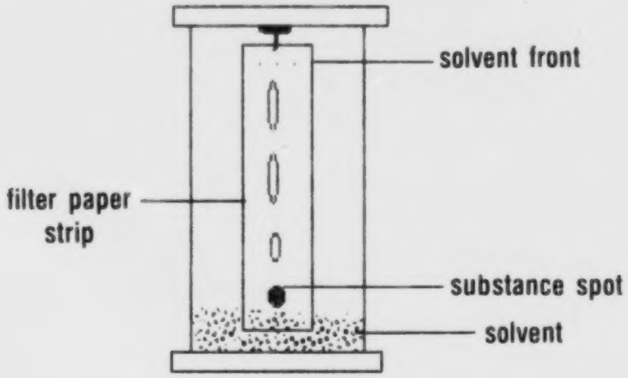


Figure 2

If the solvent is able to both ascend and descend the paper strip, it is called two dimensional chromatography. For compounds that are difficult to separate, a mixture may be separated first with one solvent, then, after the paper has been dried and rotated 90° with a different solvent. This technique allows for more complete separation of substances with similar properties. The process might go like this:

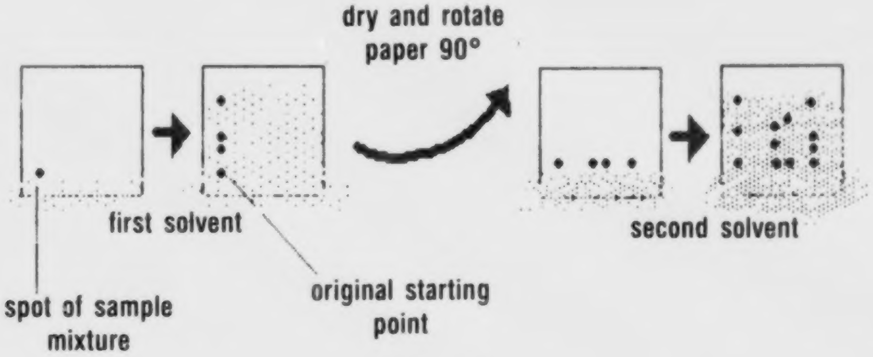


Figure 3

Applications

Melvin Calvin utilized the process of two dimensional chromatography shortly after WWII at the University of California, Berkeley, where he and other scientists were studying the biochemical steps intervening between the uptake of CO_2 and the appearance of the first complex carbohydrates in the chloroplast during photosynthesis. Calvin's solution to the problem, known today as the Calvin-Benson cycle, or the dark reactions of photosynthesis, was possible because of the development of three experimental techniques which he could use to track the carbon atom during photosynthesis.

The first technique, used to track the carbon atom of CO_2 as it becomes incorporated into other compounds, was developed as a byproduct of the project to build the first atomic bomb. The radioactive carbon isotope ^{14}C was made available to scientists, and it was then possible to prepare samples of CO_2 in which some of the carbon atoms were ^{14}C instead of the stable isotope ^{12}C usually found in nature. Any compound produced by metabolism of the radioactive material would also be radioactive.

The second technique, two dimensional paper chromatography, was an improved method for separating mixtures of different substances into their individual parts. It was a simple and yet effective method for achieving separation of the sample mixtures with which Calvin was working.

The third technique, called autoradiography, was developed to locate tiny amounts of radioactive material on two dimensional paper chromatograms (the sheets on which chromatography has been performed). The chromatogram, which has spots of ^{14}C on it, is taken into a darkroom and covered either with a type of photographic film, or with a liquid photographic emulsion. This is kept in the dark for a period of time, during which radioactive decay of ^{14}C results in the release of particles that expose the film. The exposed sections of film lie directly on the chromatogram where there were accumulations of ^{14}C containing materials, and movement of those materials can be measured. There will be an opportunity to practice autoradiographic techniques in a mineral absorption laboratory later in this manual.

Thin Layer Chromatography

It is similar to paper chromatography, except for the use of a glass plate coated with an adsorbant in the form of a loose powder or slurry. The next laboratory in this manual utilizes this process.

Ion Exchange Chromatography

It can be used in conjunction with any of the above procedures. The key difference is that ion exchange resins are installed as particles in a column, on paper, or formed as thin film on plates.

Gas Chromatography: a procedure used to analyze gases.

Gas-liquid Chromatography

The gas being analyzed adheres to a liquid film. The stationary material is a liquid spread over an inert base with a high specific surface tension, such as small diameter tubes like capillary pipets.

Gas-solid Chromatography

It is similar to gas-liquid chromatography, except that the gas is passed over a solid surface instead of a liquid one.

There exist sophisticated variations on all of the above procedures, because the chromatographic separation process has proven to be so useful. In the next laboratory we will employ one of the procedures, thin layer chromatography, to separate and identify amino acids.

Resources

Clark, B. F. and K. A. Marcker. "How Proteins Start," *Scientific American*, January 1968.

Galston, A. W., P. J. Davies and R. L. Satter. *The Life of the Green Plant*. Prentice-Hall: Englewood Cliffs, New Jersey, 1980 (3rd Edition).

Sanger, Frederick *et. al.* "Nucleotide Sequence of Bacteriophage **ØX174 DNA,"** *Nature*, 265:687, 1977.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

- autoradiography
- base line
- chromatography
- column chromatography
- effluent
- elution
- fractionation
- gas chromatography
- ion exchange chromatography
- moving phase
- origin
- paper chromatography
- stationary phase
- thin layer chromatography

Review

2. Chromatography

Name______________________________

Date______________________________

Multiple choice

______ 1. In one dimensional chromatography, each spot represents
- a) one kind of molecule
- b) a possible mix of several molecules
- c) the distance traveled by the solvent
- d) half the distance traveled by the solvent

______ 2. What radioactive isotope was used to work out the reactions of the Calvin cycle?
- a) ^{18}O
- c) ^{3}H
- b) ^{14}C
- d) ^{32}P

______ 3. Thin layer chromatography is
- a) accomplished by plastic beads in a resin column
- b) a thin layer of cotton on a glass plate
- c) a thin layer of glass surrounded by a gaseous environment
- d) a thin slurry of absorbant on a glass plate.

______ 4. Frederick Sanger accomplished the fractionation and sequencing of the amino acid components of the protein
- a) insulin
- c) PKU
- b) hydrogenase
- d) muscle

______ 5. Calvin worked out the pathway of carbon dioxide fixation using
- a) gas chromatography
- b) thin layer chromatography
- c) column chromatography
- d) paper chromatography

Matching

______ 6. Involves the use of beads or resins.

______ 7. The absorbing material must be a liquid.

______ 8. This is the passing of pure solvent down a column.

______ 9. The spot of substance to be analyzed.

______ 10. Involves the use of two or more solvents.

- a) elution
- b) two dimensional paper chromatography
- c) stationary phase
- d) base line
- e) column chromatography
- f) liquid chromatography

Essay

1. Describe the experimental techniques involved in the determination by Calvin of the first stable product of carbon dioxide fixation in photosynthesis.

2. Describe the chromatographic procedure Frederick Sanger used in the analysis of insulin.

Answers Found: p 11 - #7 & 8; p 12 - #1, 4, 6 & 9; p 13 - #2, 5 & 10; p 14 - #3; Essay 1: p 13-14; Essay 2: p 12.

3. Laboratory

Amino Acid Chromatography

When you have completed this laboratory you should be able to:

1. Describe the techniques of thin layer chromatography.
2. Determine the R*f* standards for known amino acids.
3. Use the chromatographic technique to identify unknown amino acids.

Purpose

Chromatography takes the student into a phase of science where execution is critical. Special instruments and advanced techniques now occupy the center of many life science laboratories. For example, the procedures of chromatography are not difficult to comprehend, and the chromatographic technique only yields separated compounds which must be studied by still other means. However, without careful and precise execution of chromatographic separation, further analysis would yield invalid results. Carelessness with the procedure can close potentially promising avenues of research. In coming weeks we will perform still other procedures, such as spectrophotometry and gel electrophoresis, where the watch words will have to be clean and careful.

Chromatography Process

Chromatography is an efficient tool for separating and identifying amino acids. The separation process begins by applying a tiny spot of amino acid to the base line of a matrix. In this laboratory, the matrix consists of silica gel slurry dried on a glass plate. The spotted plate is inserted vertically in a Coplin jar, and the solvent in the bottom is absorbed by the matrix in a candlewicking effect. As it moves upward, the solvent dissolves the substances in the mixture and allows them to migrate along the matrix. Different amino acids will migrate different distances. For normally colorless amino acids to be detectable through a chromatographic technique, separated compounds must be sprayed with ninhydrin which causes an oxidation reduction reaction leading to coloration.

The solvent in the bottom of the Coplin jar is carefully formulated so that the amino acids are not completely soluble in it, or they would move as fast as the solvent itself. At the same time, the substances must not be completely insoluble in the solvent, or they would not move up the plate at all. When the observer stops the procedure before the solvent reaches the top of the plate, the exact position of the solvent front (the leading edge of the area wet by the solvent) is noted. When the observer dries the finished chromatogram and sprays it with ninhydrin, the distance moved by each amino acid can be measured.

Retardation Factor

The distance moved by each amino acid is compared with the distance traveled by the solvent itself. The ratio of the two is the R*f*, or retardation factor. The R*f* expresses the ratio of the rate of movement of the migrating substance to the rate of flow of solvent within the system. In other words, the R*f* describes the ratio of distances travelled. The R*f* is always constant for a given substance in a particular solvent system.

$$Rf = \frac{\text{distance traveled by substance (mm)}}{\text{distance traveled by solvent (mm.)}}$$

The purpose of this laboratory is to determine the characteristic R*f* values of some known amino acids, and also to separate and identify unknown mixtures of amino acids using chromatographic techniques. Through chromatographing the unknown mixtures of amino acids and calculating their R*f* values, a student should be able to identify the acids by comparison with standard R*f*s.

Pre-lab

Supplies needed for amino acid chromatography:

Equipment

Coplin jar
capillary pipets, 10 ml.
fume hood (or other separate area)
thin layer chromatography sheets
pencil

Materials

amino acid standards, 20
amino acid unknowns (3 unlabelled amino acids and 2 mixtures)
chromatography solvent
ninhydrin spray
petroleum jelly

Some of these materials have to be made in advance, and some can only be ordered from a limited number of suppliers.

Special Preparations

1) Amino acid standards kits: it is best to store the amino acid solutions in small vials which will preserve them for several years. Standard solutions usually contain 0.2% of each amino acid in 10% propanol. When preparing for use, dissolve 20 mg. of each amino acid solution in 9 ml. distilled water and 1 ml. propanol.

2) The teacher will select the unknowns and make mixtures from the stock of standards. Number per the chart at the end of this laboratory.

3) Chromatography solvent: butanol — acetic acid — water (4:1:1). Mix 160 ml. butanol, 40 ml. acetic acid, and 40 ml. water. Keep tightly covered and provide fresh solvent for each laboratory.

4) To use aerosol ninhydrin spray, set up a spray area in a fume hood, in a large cardboard box, or outside to keep the fumes out of the general laboratory area.

Time Required:

The amino acid chromatography laboratory requires three classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the following: pencils, chromatography sheets, amino acid standards, unknown amino acids, and capillary pipets.

Steps

A. Place a chromatography sheet flat on a table, silica gel side up. Touch it as little as possible to prevent contamination by skin oils. Measure 1 cm. up each short side with a pencil. Make a small dot on each side and join them with a straight light pencil line across the face of the sheet. The long edge of the sheet containing the line will now be known as the origin, and the line known as the base line.

B. Carefully spot the amino acid standards and unknowns along the base line. Spot them no closer than 2 cm. apart. Otherwise, they tend to bleed into each other. Each plate will take five spots. This is how it is done:

1) use a separate capillary pipet for each amino acid solution and unknown;

2) touch the gel very quickly with only the end of the pipet so that the spot will be the smallest possible;

3) let the spots air dry thoroughly;

4) while the first chromatography sheet is drying, make the others required (each student or laboratory group should use five chromatography sheets in order to spot all 25 of the amino acids and unknowns);

5) label the sheets sequentially with pencil on the very top right hand side of each sheet;

6) when the first spots dry, apply a second spot in the exact same place, and of the exact same amino acid or unknown (this intensifies the amino acid concentration in a small area); and,

7) allow these second spots to dry thoroughly.

C. Record the name of the amino acid or the number of the unknown below each of the spots indicated on the following chromatography sheet diagrams:

1.

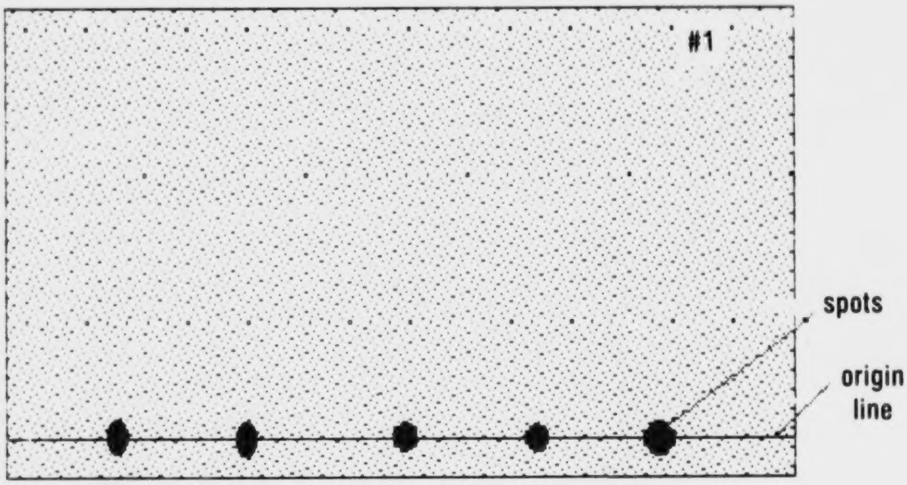


Figure 1

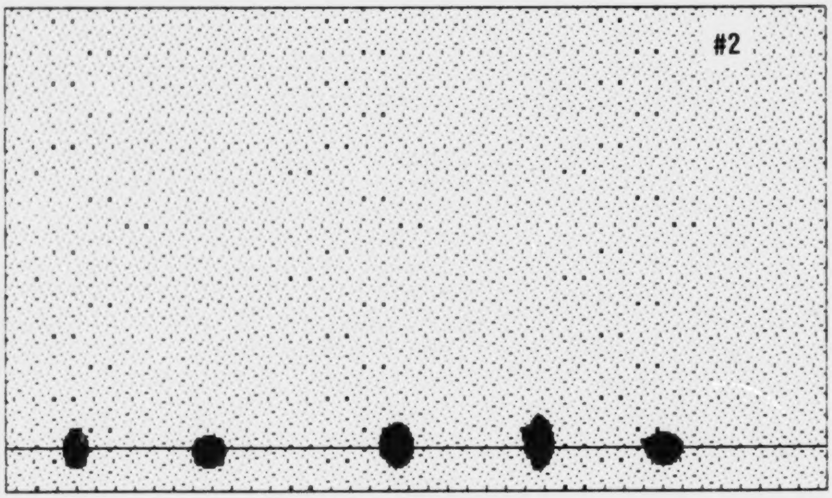


Figure 2

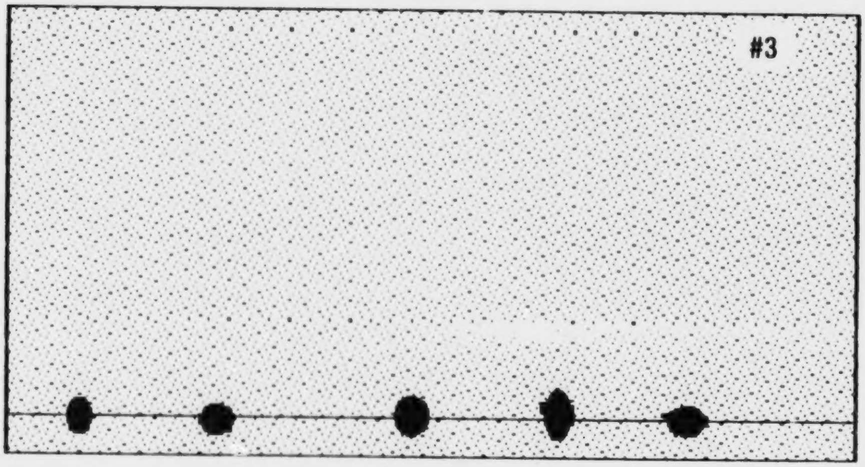


Figure 3

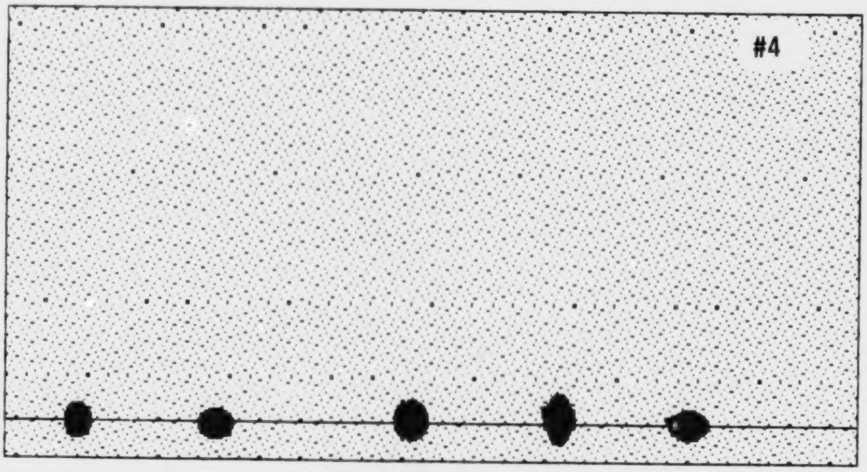


Figure 4

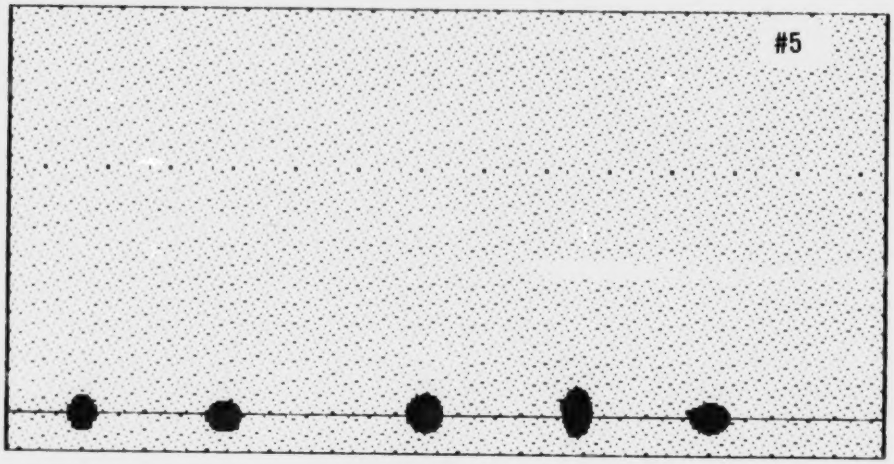


Figure 5

Procedure: Hour 2

Students need the following: spotted and dried chromatography sheets, Coplin jar and lid, solvent, and petroleum jelly.

It is now time to chromatograph.

Steps

A. Very carefully set the chromatographic sheets inside a Coplin jar containing solvent. The solvent level should be low enough so that the base line and spots are above, not in, the solvent. Do not allow the sheets to touch each other in the jar.

B. Place the lid on the Coplin jar after first placing a heavy layer of petroleum jelly around the lip of the jar. This helps seal the solvent fumes inside the jar.

C. Let the solvent move up the gel until it is within 1 cm. of the top. This can take anywhere from 30 minutes to two hours, depending upon the environment (temperature, air pressure, and humidity all affect chromatographic adsorption). Students will have to make arrangements to complete this phase of the laboratory outside of class if the process takes longer than a class hour.

D. When the solvent is 1 cm. from the top of the sheets, stop the experiment by removing the sheets from the Coplin jar. Immediately lightly pencil or scratch an area on each side of the sheet above each spot to show the position reached by the solvent front.

E. Air dry the sheets overnight. Allow nothing to touch them while they are drying.

Procedure: Hour 3

Students need the following: prepared chromatography sheets, ninhydrin spray, and a fume hood (or a temporary fume hood constructed of a cardboard box, or an outdoor area in which to spray the ninhydrin).

Be extremely careful when using ninhydrn. It is toxic. Do not breathe it or get it on your skin. If you are accidentally contaminated with ninhydrin, wash the area thoroughly with lots of water.

Steps

A. Carefully prop the chromatography sheets under the fume hood, or in a safe outside area, and spray them with ninhydrin. Ninhydrin reacts with amino acid spots to produce identifiable visible spots.

B. For each spot, record the color in the chart on page 22. The amino acids having primary ∂-amino groups yield pink to purple spots; the secondary amines such as proline and hydroxyproline yield yellow spots. Recording the color of the ninhydrin sprayed amino acid gives the student a crude check on R*f* calculations used to identify unknowns.

C. Measure the distance the spot traveled. The precise distance is the distance from the center of the original spot, at the origin, to the center of the colored spot. R*f* generally decreases with increasing molecular weight. However, in the series glycine, alanine, valine, and leucine, the larger the alkyl group, the more the acid tends to move along with the organic solvent, because of its increased solubility.

D. Also measure the exact distance traveled by the solvent in each case (solvent movement can be irregular across the face of an entire plate: correct results require solvent front measurements immediately above each spot).

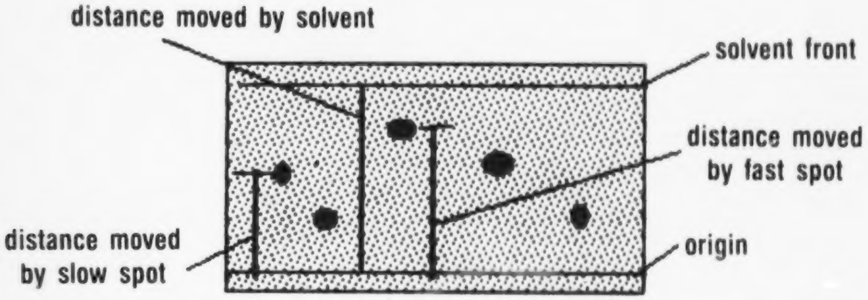

distance moved by solvent
solvent front
distance moved by fast spot
distance moved by slow spot
origin

Figure 6

2. Chart results in figure seven as follows:

Standard Amino Acids	Color	Distance Amino Acid	Distance Solvent	R*f*
Alanine				
Arginine				
Asparagine				
Aspartic Acid				
Cysteine				
Glutamic Acid				
Glutamine				
Glycine				
Histidine				
Isoleucine				
Leucine				
Lysine				
Methionine				
Phenylalanine				
Proline				
Serine				
Threonine				
Tryptophan				
Tyrosine				
Valine				
Unknown 1				
Unknown 2				
Unknown 3				
Mixture 1				
Mixture 2				

Figure 7

3. Now the amino acids in the unknowns and mixtures can be identified by comparing the R*f* of each spot with the R*f* values for the standard amino acids. What are the unknowns?

Unknown #1 ____

Unknown #2 ____

Unknown #3 ____

Mixture #1 ____________________ and ____________________

Mixture #2 ____________________ and ____________________

Resources

Baker, Jeffrey J. W. and Garland E. Allen. *Matter, Energy and Life: An Introduction to Chemical Concepts*. Addison Wesley: Reading, Massachusetts, 1981 (4th Edition).

Phoenix Films. *Chromatography Techniques: Amino Acids*. Film Loop #81-6025, color, 3 minutes 40 seconds.

Windholz, M. et. al., Eds. *The Merck Index*. Merck and Co.: Rahway, New Jersey, 1976 (9th Edition).

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

Coplin jar
ninhydrin
retardation factor (R*f*)
solvent front

Review

3. Amino Acid Chromatography

Name________________________________

Date________________________________

Multiple choice

______ 1. The most often used chromatographic process for amino acid analysis is

a) thin layer
b) gas
c) column
d) ion exchange

______ 2. The R*f* standards of amino acids refer to

a) the reference index for amino acids
b) their ready facets of protein acceptance
c) their retardation factors
d) the reference points in protein analysis

______ 3. The secondary amines such as proline and hydroxyproline yield

a) yellow spots
b) green spots
c) pink spots
d) blue spots

______ 4. You apply the sample material to be tested in thin layer chromatography to the

a) solvent front
b) effluent
c) top edge of the glass
d) base line

______ 5. What chemical reacts with amino acid spots to produce identifiable visible spots?

a) petroleum jelly
b) ninhydrin
c) butyl alcohol
d) all primary ∂ amino groups

Matching

______ 6. Increasing molecular weight

______ 7. Increased solubility

______ 8. Solvent

______ 9. Glycine

______10. Sealant

a) pink spots

b) ∂ amino groups

c) larger alkyl groups

d) butyl alcohol

e) R*f* decreases

f) petroleum jelly

Essay

1. Discuss the role of solvent in the different types of chromatography.

2. Discuss the different factors which might influence the rate, regularity, and direction of amino acid spread on a thin layer chromatography plate.

Answers Found: p 17 - #1 & 2; p 18 - #4; p 21 - #3, 5, 6, 7, 8, 9, & 10; Essay 1: p 17; Essay 2: p 17.

4. Discussion

Polarimetry

When you have completed this discussion you should be able to:

1. Describe polarized light.
2. Sketch and describe the function of a polarimeter.
3. Define optical activity and provide examples.

Scientists discovered early in the nineteenth century that solutions of sugar and certain other naturally occurring chemicals rotate a beam of polarized light passing through the solution. They called such substances optically active, a term still in use. The instrument used to demonstrate or to measure this rotation was given the name polarimeter.

Optical activity is best understood by knowing how a polarimeter works. And to understand a polarimeter, one must know about the polarization of light. When these are comprehended, the optical activities of some organic compounds can be studied.

Polarized Light

Physicists describe visible light as a form of electromagnetic radiation. There are many kinds of electromagnetic radiation, such as X-rays, ultraviolet light, and radio waves. All these forms of energy travel in the form of waves in which a matched pair of electric and magnetic fields move up and down as the wave moves forward. This movement is at the speed of light and is conveniently depicted by a sine wave such as the following.

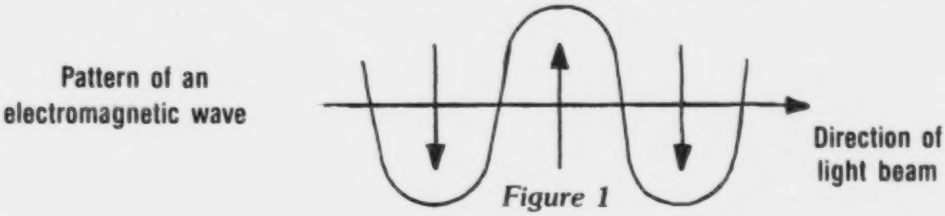


Figure 1

A beam of light consists of an infinite number of such light waves moving in every possible direction. If one were to look at a beam of light directly, the light beams would scatter in all directions from the original source of light. This is called nonpolarized light. In a single flat plane, it would look something like this:

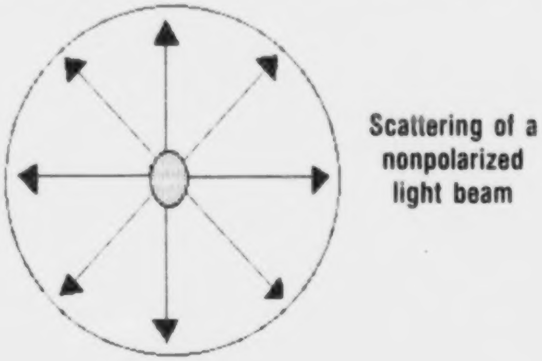


Figure 2

This property of light can be altered. A light beam can be reflected off a sheet of glass in such a way that it is plane-polarized, that is, the waves are made to move parallel to each other. Schematically, light waves moving all in the same direction might look something like this:

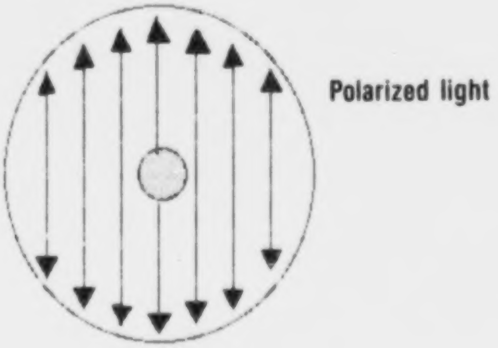


Figure 3

There is another way to polarize a beam of light. Certain crystals can convert nonpolarized light into plane-polarized light. Crystals of the mineral calcite are structured in such a way that they naturally polarize light. The material known as polaroid is an artificial substance that does the same thing.

The Polarimeter

The polarimeter is a simple device designed to study compounds that affect the polarization of light. This is how a polarimeter is designed:

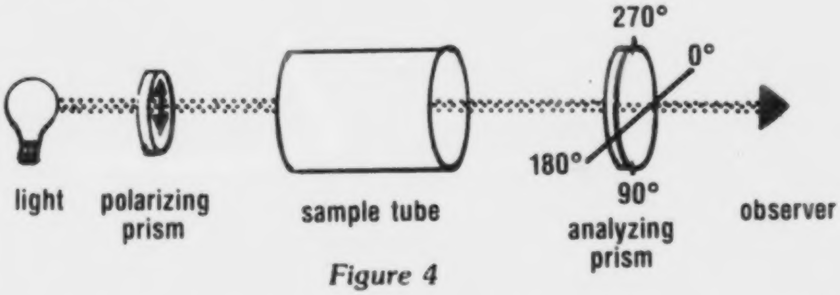


Figure 4

The light source is directed at a polarizing prism, which is made of calcite, polaroid, or any other polarizing material. The light is polarized in the prism and leaves the prism plane-polarized. The light then enters the sample tube which contains a liquid sample. If the tube is filled with nothing but distilled water, the plane-polarized light will pass through without a change of direction. If the analyzing prism, which is also polarized, is lined up at the same angle as the polarizing prism, all of the polarized light rays will pass through. However, if the sample changes the direction of the light passing through the tube, fewer light rays emerge from the analyzing prism. A difference of ninety degrees between the direction of the polarized light and the analyzing prism yields darkness.

The observer turns the analyzing prism slowly until the maximum amount of light shines through the analyzing prism. Since the analyzing prism is graduated, the observer can read the angle of rotation. Both the extent and direction of rotation can be read. The result usually is recorded as plus (+) or minus (−) the angle of rotation. A solution which rotates light 20° to the left would be recorded as −20° and would be called levorotatory, since the prefix levo means "left" in Latin. Solutions which rotate light to the right are called dextrorotatory.

Background

Polarimeters traditionally were built around carefully cut prisms formed from Iceland Spar (calcite). Such prisms, known as Nicol prisms, were often found in advanced chemistry courses and were used only by faculty and a few graduate students. They were too expensive and delicate for general use. For many years, developmental work on polarimeters was focused on making instruments that were more and more precise. The 360° measuring scales can be equipped with special eyepieces and verniers that can be read to the nearest 0.01°. The sample tubes also underwent a similar development so that the length of tube through which the polarized light passes can be read to the nearest 0.01 mm.

Expensive and complicated polarimeters are used when high degrees of accuracy are required. There are many situations in which a general result obtained with a simplified polarimeter would be sufficient. Some of these applications are industrial, others are academic. For example, a simple polarimeter is all that is required to make quick checks of whether organic compounds ranging from carbohydrates to amino acids are optically active. This need was met by development of low cost polarimeters.

A number of engineering changes were incorporated into these new generation polarimeters. For instance, measurements are made at the point of optical extinction rather than the point of maximum brightness. Some small degree of accuracy was sacrificed so that eye strain associated with maximum brightness can be eased. Also, these polarimeters use a polaroid sheet instead of expensive crystal prisms.

Optical Activity

Molecules which rotate a beam of polarized light are described as being optically active. Chemists wanted to know if an optically active molecule has a typical molecular structure, and, if the molecular structure is known, if its optical activity can be predicted. In 1874, two chemists, the Frenchman LeBel and the Dutchman van't Hoff, independently solved the problem. They noted that the four valence elecrons in a carbon atom are normally directed at the four corners of a tetrahedran. If one were to construct a tetrahedral carbon atom with four different substituents at the four corners, it is possible to make two **different** models of the figure. It was learned that a molecule is optically inactive if its mirror image is superimposable. On the other hand, a molecule is optically active if its mirror image is **not** superimposable.

A simple example of mirror images which are not superimposable is human hands. They are mirror images of one another, but with both palms facing up, they do not fit one upon the other. It is still not understood why molecules which are mirror images but not superimposable are optically active.

Enantiomers

Two molecules which are nonsuperimposable mirror images of each other are called enantiomers. Since enantiomers are exactly identical in their chemical properties, usual chemical reactions yield as many molecules of one type of enantiomer as another. This 50-50 mixture of two optically active forms of the same molecule is called a racemic mixture. In racemic mixtures there is no optical activity, since one half of the molecules rotate light to the left and one half rotate light to the right. The sum total of light rotation is zero.

Louis Pasteur, the "Father of Microbiology", discovered that an enantiomer sometimes crystallizes in a slightly different form from its mirror image. He used a magnifying glass to painstakingly pick out one enantiomer from the other. Unfortunately, only a few optical isomers show a difference in crystalline structure, and this method is not used today. Some chemical reactions can be designed to yield specific enantiomers. The largest source of enantiomers is microorganisms which produce enantiomers as metabolic byproducts. Scientists let these microorganisms do the work for them, although they do not yet fully understand why microorganisms produce specific enantiomers.

Excepting their structure, enantiomers have the same physical and chemical properties. There are only two ways in which they differ one from the other, other than their form. They rotate the plane of polarized light, and they are used differently by living organisms. For example, dextrorotatory glucose (D-glucose) is extremely important in human metabolism. It is formed during the metabolism of starch and sucrose and is broken down by our bodies to produce carbon dioxide, water, and energy. Levorotatory glucose (L-glucose) cannot be used by the human body at all, even though it has the same sweet taste. We would starve to death on a diet which contained L-glucose as the source of energy.

Diasteriomers

Sometimes a molecule is not composed in merely two enantiomeric forms, but three, or even more. They are said to be compounds with more than one asymmetric carbon atom. An example of this is tartaric acid:

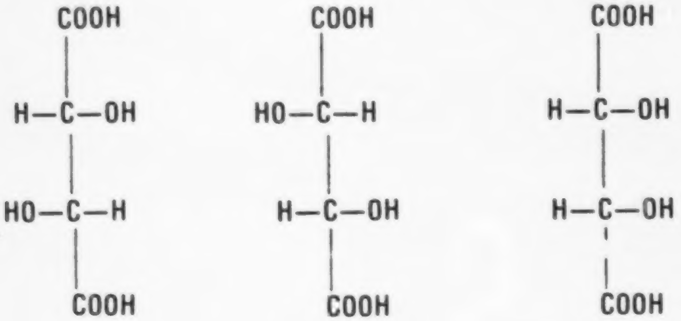
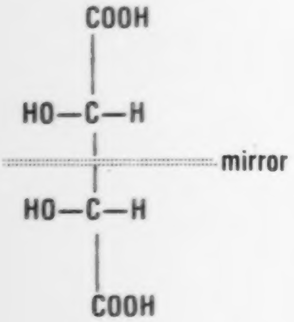


Figure 5

The first and second isomers are mirror images of each other and are enantiomers. The first and second isomers are not mirror images of the third isomer, and so they are termed diasteriomers of each other. The third isomer has its mirror image too:



Tartaric acid has a special property known as internal symmetry. The upper halves are identical to the lower halves. If a molecule possesses this internal symmetry, there is only one optically active form of that form of the compound. That isomer is referred to as the meso form of the compound.

Figure 6

Polarimeter Demonstrations

The capabilities of the polarimeter may be demonstrated by any of the following experiments. The instructor may wish to perform these demonstrations and discuss the conclusions drawn from them. Or, students may wish to proceed with them as experiments, submitting their results to the instructor in formal laboratory reports.

Qualitative Polarimetry: Carbohydrates

The polarimeter easily demonstrates how different carbohydrates rotate polarized light. Many optically active sugars are readily available and relatively low in cost. Examples are D-glucose and L-glucose. The following demonstration can be conducted with standard 35 ml. polarimeter cells, or tubes.

Weigh 5 grams of sugar and transfer it to a 25 ml. volumetric flask. Dissolve the sample sugar in water and dilute it to the 25 ml. calibration mark on the volumetric flask.

Pour the solution into the polarimeter tube, dry the outside, and carefully position the tube in the polarimeter. Turn on the polarimeter light and rotate the eyepiece until the light is extinguished. This will happen near the 0 degree mark. With some substances having high rotation, some secondary color effects may be noticeable. If this happens, choose the darkest point, the point where there is neither a greenish nor a reddish cast. Make several readings and average results.

Remove the polarimeter tube and measure and record, in decimeters, solution height from the inside of the base to the bottom of the meniscus. Empty, clean, and dry the cylinder.

Calculate the specific rotation as follows, remembering that if the rotation is clockwise, rotation is plus, and if counter clockwise, rotation is minus:

$$\text{specific rotation} = \frac{\text{observed rotation} \times \text{ml. of solution}}{\text{solution height (dm.)} \times \text{grams of unknown}}$$

If the instructor or students repeat this experiment with different optically active sugars, the specific rotations of each sugar can then be compared.

Qualitative Polarimetry: Amino Acids

With the exception of glycine, all amino acids are optically active. However, many exhibit rather low specific rotations, and it is best to demonstrate the polarimeter with those amino acids having large specific rotations.

L-cystine is a good amino acid to use, since it is less expensive than some and has a relatively large specific rotation. Dissolve 0.25 grams of L-cystine in 15 ml. of 1 molar hydrochloric acid, and transfer the solution to a 25 ml. volumetric flask. Add additional acid until the 25 ml. mark is reached.

Proceed as per the demonstration on carbohydrates in qualitative polarimetry.

Changing the concentration of the hydrochloric acid solvent changes the specific rotation of the amino acid solute. The instructor may wish to have students experiment with different solvent concentrations.

Quantitative Polarimetry

The extent of light rotation is also dependent upon the concentration of the optically active substance. The polarimeter can be used to determine the concentration of a solution containing optically active material. This is done by making up a series of solutions of different known concentrations of the material, measuring their observed rotations, plotting a graph, and determining an unknown concentration by comparing its observed rotation with the graph.

Make 25 ml. of solution as per the carbohydrate demonstration on qualitative polarimetry, and measure and record its rotation. The solution is unaffected by use in the polarimeter and can be repeatedly diluted to produce solutions of new concentration. This procedure is called serial dilution.

Take half of the original solution and dilute it with an equal amount of water or solvent. Calculate the new concentration and measure and record its specific rotation. Continue making such dilutions and measuring their rotations until there are at least five readings (for further instruction on serial dilution see Laboratory #9).

Plot a graph of observed degrees of rotation against concentration. Measure the rotation of a solution of unknown concentration, and determine its concentration by reference to the graph.

The following inverse proportion can be used to determine the concentration of the diluted solution:

$$\frac{\text{concentration of diluted solution}}{\text{concentration of concentrated solution}} = \frac{\text{volume of concentrated solution}}{\text{volume of diluted solution}}$$

Resources

Calvin, M. *Organic Chemistry of Life: Readings from Scientific American.* W. H. Freeman: San Francisco, 1973.

Hughes, R. E. "Human Dietary Patterns and Technological Change," #17. *Oxford Biology Readers.* Oxford University Press: London, 1971.

Scrimshaw, Nevin S., and Lance Taylor. "Food," *Scientific American*, September 1980.

Sharon, Nathan. "Carbohydrates," *Scientific American*, September 1980.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

calcite
dextrorotatory
diasteriomer
electromagnetic radiation
enantiomer
levorotatory
meso form
optical activity
polarimeter
polarization
polaroid
racemic mixture
serial dilution

Review

4. Polarimetry

Name ______________________________

Date ______________________________

Definitions

1. Optical activity ______________________________
2. Racemic mixtures ______________________________
3. Polarized light ______________________________
4. Nonsuperimposable mirror images ______________________________
5. Isomers ______________________________

Short answer

1. Make a sketch of a polarimeter, labeling each part of the instrument. Explain what happens to a beam of light that passes through it.

2. Using tartaric acid, draw a pair of enantiomers. Draw their diasteriomer and its meso form. Label your drawings.

Answers Found: p 27 - #3; p 29 - #1, 2, & 4; p 30 - #5; Essay 1: p 28-29; Essay 2: p 30.

5. Discussion

Spectrophotometry

When you have completed this discussion you should be able to:

1. Explain the wave model of light.
2. Explain the photon model of light.
3. Explain how living things depend on light.
4. Explain how a spectrophotometer works.

Particle Theory of Light

Over 300 years ago, Sir Isaac Newton, the English physicist, separated visible light into a spectrum of colors by passing it through a prism. When he passed the spectrum of colors through a second prism white light was produced once again. By this experiment, Newton showed that white, or visible light, is actually made up of a number of different colors. Their separation is possible because light of different colors is bent at different angles when passing through the prism. Newton believed that light consisted of a stream of particles (he termed them "corpuscles"), partly because of their tendency to travel in a straight line.

Wave Model of Light

James C. Maxwell, in the nineteenth century, demonstrated that what we experience as light is in truth a very small part of a vast, continuous spectrum of radiation, the electromagnetic spectrum. As Maxwell showed, all the radiation included in this spectrum act as if they travel in waves. The wavelengths (the distances from one wave peak to the next) range from those of gamma rays, which are measured in nanometers, to those of low frequency radio waves, which are measured in kilometers. Within the spectrum of visible light, red light has the longest wavelength and violet the shortest. All these radiations travel 300,000 kilometers per second (or 186,000 miles per second) in a vacuum. This is the speed of light. The chart below demonstrates that visible light makes up only a small portion of the electromagnetic spectrum:

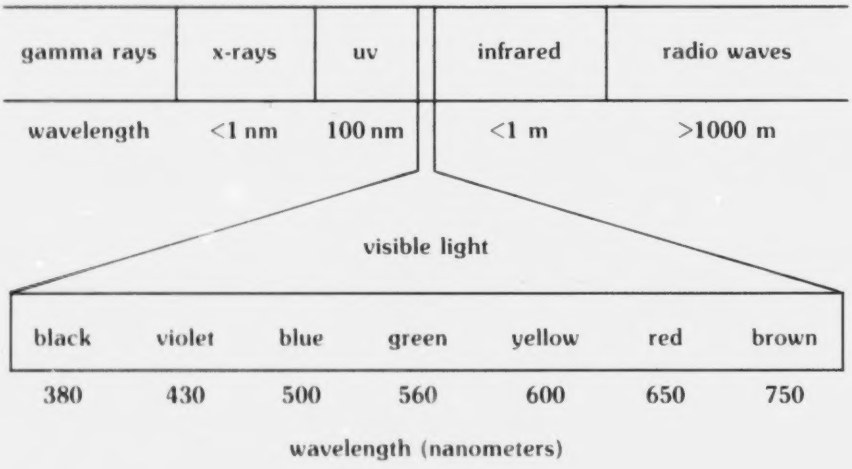


Figure 1

The wave model of light led to predictions that the brighter the light the greater the force with which electrons would be dislodged from metals being struck by light. However, whether or not light can eject the electrons of a particular metal depends not on the brightness of the light but on its wavelength. It had become clear by 1900 that the wave model of light was not adequate to explain this phenomenon. For example, when a zinc plate is exposed to ultraviolet light, it acquires a positive charge. The metal becomes positively charged when light energy dislodges electrons, forcing them out of the metal atoms. This photoelectric effect can be produced in all metals. Every metal has a critical wavelength for the effect; the light (visible or invisible) must be of the correct wavelength, or shorter, for the effect to occur. The critical wavelength of some metals, such as potassium, selenium, and sodium, is within the spectrum of visible light. Therefore, visible light striking the metal can set up a moving stream of electrons. Such a moving stream of electrons is an electric current. Burglar alarms, exposure meters, television cameras, and electric eye door openers all operate on this principle of turning light energy into electrical energy.

Photon Model of Light

To explain this phenomenon, the particle model of light, as originally advanced by Sir Isaac Newton, was resurrected by Albert Einstein in 1905 as the photon model of light. Albert Einstein stated that light is composed of particles of energy which he called photons. The energy of a photon is not the same for all kinds of light. It is inversely proportional to the wavelength. Phrased differently, the longer the wavelength the lower the energy. Therefore, photons of violet light, the shortest visible light wavelength, have almost twice the energy as photons of red light, the longest visible wavelength.

The wave model of light permits physicists to describe certain aspects of its behavior mathematically, and the photon model permits another set of mathematical predictions and calculations. The two models of light are considered today to be complementary light models, each explaining an aspect of light. We still lack an integrated theory to completely describe the phenomenon called light.

Light and Living Things

To understand light and its action on living things is a very important aspect of biology. Visible light is responsible for photosynthesis (on which all life depends), vision (on which many organisms depend), and the rhythmic day and night aspects of many biological activities. George Wald of Harvard University thinks that if life exists elsewhere in the universe, it is probably dependent on the same small fragment of electromagnetic radiation, visible light. Wald bases his conjecture on two points.

First, living things are composed of large, complicated molecules held together by energy bonds. Radiation of even slightly higher energies than the energy of violet light, that is, radiation at a frequency just below visible light, tends to break these bonds and disrupt the structure and function of the molecules. These lower frequency, higher photon energy level radiations drive electrons out of atoms. On the other hand, higher frequency radiations beyond visible light, ones with lower photon energy levels than that of red light, are absorbable by water. Since water makes up the great bulk of all living things, radiation higher in frequency than visible red can transfer its energy in the form of heat to the water within the living organism. In sufficient doses, at frequencies either above or below visible light, electromagnetic radiations are lethal to life. Only those radiations within the range of visible light have the property of exciting molecules (moving electrons into higher energy levels) without destroying them.

Second, visible light provides the bulk of electromagnetic radiation available in the biosphere. Much of the rest of the spectrum is screened out by the ozone layer, water vapor, and carbon dioxide before it reaches the earth's surface. Quite simply, the bulk of the energy available at the surface is precisely that which can be used by living organisms, visible light.

Colors

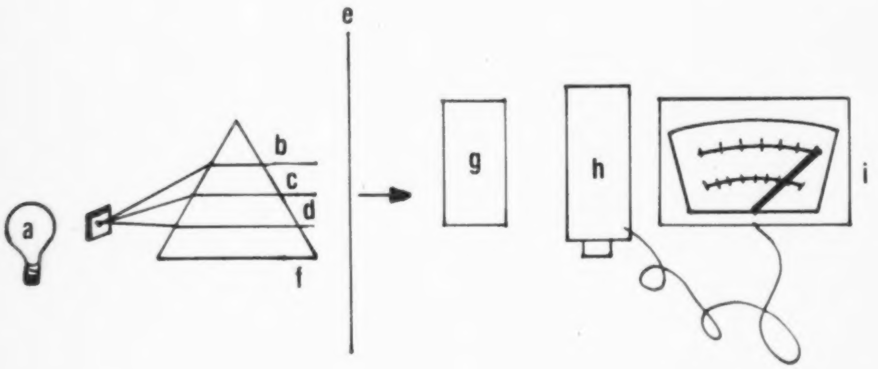
Man's perception of visible light is in the form of full spectrum white light, or as colors. Colors are determined by the reflection of unabsorbed frequencies. Thus, a red appearing object is one which absorbs the blue and or green components of visible light. A colored object selectively absorbs and reflects particular frequencies within the visible light spectrum.

Spectrophotometry

Man's perception of color is only qualitative. It indicates what is happening, but it says nothing about the extent of the event taking place. Spectrophotometry, on the other hand, is the quantitative study of visible light. Spectrophotometers are instruments which electronically quantify the amount and kinds of light absorbed by molecules in solution. The instrument does this by measuring the relative amounts of radiant energy as a function of wavelength (frequency).

In its simplest form, a spectrophotometer has a source of white light focused on a prism or diffraction grating which separates the white light into disparate bands of radiant energy. Each wavelength (color, or frequency) is then selectively focused through a narrow slit by setting the machine at the appropriate wavelength. The width of this slit is important to the precision of the measurement; the narrower the slit, the more precisely a specific wavelength of light is selected. This single wavelength, called the incident beam (I_0), passes through the sample being measured.

The sample, usually dissolved in a suitable solvent, is held in a cuvette. The cuvette is an optically precise, rectangular, glass container standardized to have a light path one centimeter across. After passing through the sample, the selected wavelength of light, now called the transmitted beam (I), strikes a photoelectric tube. If the substance in the cuvette has absorbed any of the incident light, the transmitted light will then be reduced in total energy content. When the transmitted beam strikes the photoelectric tube, it generates an electric current proportional to the intensity of the light energy striking it. By connecting the photoelectric tube to a device that measures electric current (a galvanometer), a means of directly measuring the intensity of the transmitted beam is achieved.



a = white light
b = red
c = green
d = blue
e = exit slit
f = refracting prism
g = sample cuvette
h = photoelectric tube
i = galvanometer

Figure 2

The typical spectrophotometer has a galvanometer with two scales, one indicating the percent transmittance (%T, or, the amount of light that has passed through the sample), and the other the absorbance (A, or, the amount of light absorbed by the sample). Absorbance is measured on a logarithmic scale with unequal divisons graduated from 0.0 to 2.0. Absorbance can also be referred to as optical density (O.D.)

Because most organic molecules are dissolved in a solvent before measurement, a source of error arises in that the solvent itself may absorb light. It is necessary therefore to "subtract" the absorbance of the solvent. To achieve this, a "blank" (the solvent without the sample to be studied) is first inserted into the instrument, and the scale is set to read 100% transmittance (or 0.0 absorbance) for the solvent. The "sample", containing the solute plus the solvent, is then inserted into the instrument. Any reading on the scale that is less than 100% transmittance (or greater than 0.0 absorbance) is considered to be due to absorbance by the solute only.

Spectrophotometers are not limited to detecting only absorption of visible light. Some are adaptable to function with a source of ultraviolet light, such as a hydrogen lamp or a mercury lamp, which can provide wavelengths that range from 180 to 400 nanometers. These ultraviolet wavelengths are especially useful in studying such molecules as amino acids, proteins, and nucleic acids, because these molecules have characteristic absorbances in the ultraviolet range. There are even spectrophotometers with sources of infrared radiation which provide wavelengths from 780 to 25,000 nanometers. There also are instruments available which can continuously give the desired ratio between sample cuvette and blank cuvette, both visually and on a strip chart recorder, although these are not generally found in introductory biology laboratories.

The typical spectrophotometer has the following parts:

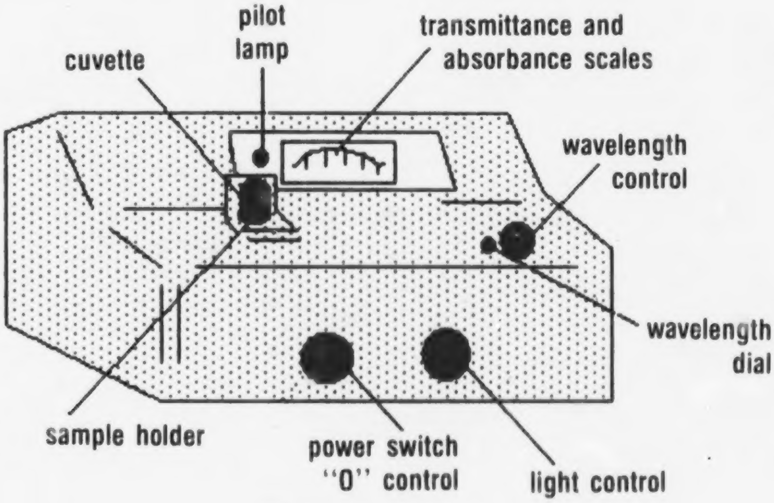


Figure 3

The spectrophotometer is an extremely useful instrument with which to study biological molecules and processes. For instance, the spectrophotometer was used to analyze the molecular reactions whereby chlorophyll converts light energy into chemical energy, and, in the process, it revealed the existence of different kinds of chlorophyll. Detailed analysis of enzymatically aided reaction sequences within organic compounds is possible using the spectrophotometer. The instrument also is used to study hormones associated with day and night rhythms in animals. In two subsequent laboratory activities, enzyme activity and photosynthesis, the research applications and usefulness of results yielded by the spectrophotometer will be demonstrated.

Resources

Bauman, R. P. *Absorption Spectroscopy*. John Wiley & Sons: New York, 1962.

Nassau, Kurt. "The Causes of Color," *Scientific American*, October 1980.

Van Norman, R. W. *Experimental Biology*. Prentice-Hall: New York, 1971 (2nd edition).

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

absorbance
cuvette
electromagnetic radiation
galvanometer
incident beam (I_0)
light wavelength
photoelectric cell
photon
spectrophotometer
transmittance
transmitted beam (I)
visible light

Review

5. Spectrophotometry

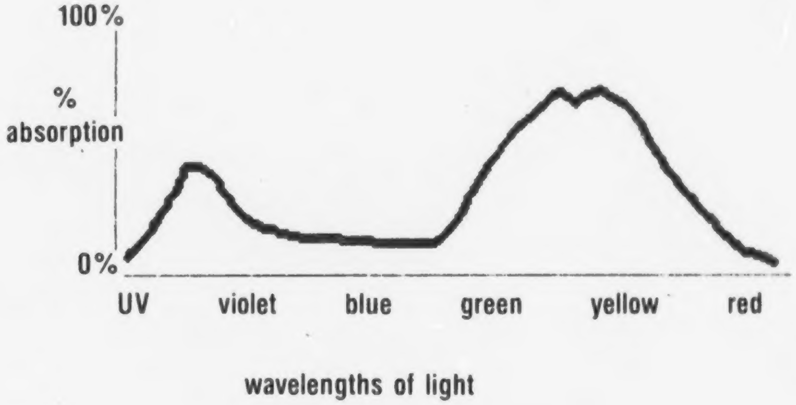
Name______________________________

Date______________________________

True or false

______ 1. The wavelengths of light responsible for the green color in leaves are in the green range of visible light.

Questions 2 through 5 are based on the following chart. The graph represents the absorption spectrum of a plant pigment.



______ 2. The pigment will appear yellowish green.

______ 3. The pigment will appear red.

______ 4. The pigment will appear colorless.

______ 5. The pigment will appear purple-violet

Essay

1. Compare the wave model of light with the photon model of light.

1. Describe how a spectrophotometer works. In your discussion diagram the parts of a typical spectrophotometer.

3. Why are living things so dependent upon visible light in the spectrum of electromagnetic radiation?

4. How do photoelectric cells work?

Answers Found: p 36: #1 - 5. Essay 1: p 35 - 36; Essay 2: p 37 - 38; Essay 3: p 36; Essay 4: p 35

6. Exercise

The Atomic Spectrum of Hydrogen

When you have completed this laboratory you should be able to:

1. Explain the quantum theory.
2. Calculate the ten lowest energy levels of hydrogen.

Purpose

Atoms, depending upon the energy level at which they exist at the moment, will emit specific wavelengths of light. These emissions, if properly measured and calculated, tell the observer about the energy levels of the atoms. These energy levels are called quanta, because they are multiples of discrete energy units, the photons referred to in discussion five. Energy levels for atoms of every element are predictable, and so any observed line in the spectrum of an element is equatable to a particular energy level. The spectrophotometer can accurately measure these light emissions. In this exercise you will calculate hypothetical hydrogen energy levels and then compare these with actual figures derived from spectrophotometer readings of hydrogen.

Background

Quantum Theory

The quantum theory, which states that atoms and molecules can exist only in specific states, each of which has a fixed amount of energy, was developed from the particle model of light, first advanced by Sir Isaac Newton and resurrected by Albert Einstein. The particles of light energy, which Einstein called photons, are inversely proportional to the wavelength of light. When an atom or molecule changes its state, it must absorb or emit an amount equal to the difference between the energy of the initial and final states. This energy may be absorbed or emitted in the form of light and can be represented by the following equation:

$$\Delta E = \frac{hc}{\lambda}$$

where ΔE is the absolute value of the change in energy in ergs (an erg is a unit of work equal to the force needed to push a one gram mass one centimeter in one second),

h is Planck's constant (named after Max Planck who first discovered this relationship) which is 6.6251×10^{-27} erg / second,

c is the speed of light, 3.00×10^{10} cm. / second, and

λ is the wavelength in cm.

The change in energy, ΔE, of the atom or molecule is positive, if light is absorbed, and negative, if it is emitted.

Sodium Energy Levels

Let us consider a specific application of the above equation. On the left side of the following chart, the sodium atom is shown to exist at two low energy levels (the right side of the chart will be discussed later). Ground state, for example, occurs at 8.25×10^{-12} ergs below the energy the atom has when it ionizes, which is assigned the arbitrary value of zero for all elements. The energy levels, as can be seen, are shown as negative, with the lower energy level having the more negative value.

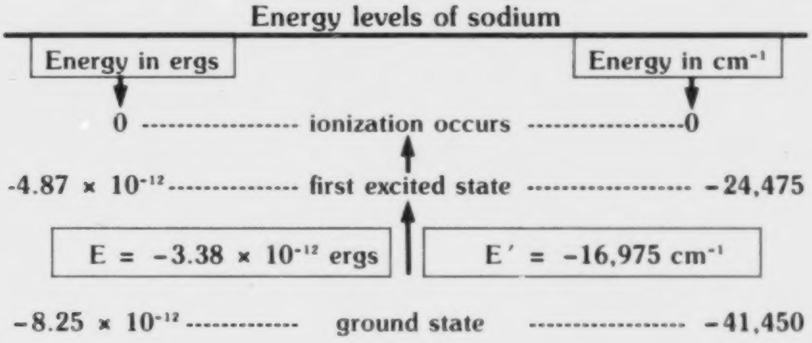


Figure 1

A sodium atom ordinarily exists at its lowest possible energy state, which is termed the ground state. If the atom is excited to its next higher state, it will tend to be unstable and if possible make a transition back down to its ground state. The energy of the atom will decrease by about 3.38×10^{-12} ergs while making the downward transition. This amount of energy may be radiated as light, which will have a wavelength represented by the equation, $\Delta E = \frac{hc}{\lambda}$, also known as Planck's law.

Using Planck's law, the wavelength of light emitted by sodium jumping from the first state back to the ground state is 5.88×10^{-5} cm. Wavelengths of light are normally stated in angstroms (Å). Since Å equals 1×10^{-8} cm., the sodium light wavelength is 5,880 Å. Atomic spectra arise from transitions of this sort, and the associated wavelengths can be determined just as in the following calculations for sodium.

$$\lambda = \frac{hc}{\Delta E} = \frac{6.625 \times 10^{-27} \times 3.00 \times 10^{10}}{3.38 \times 10^{-12}} = 5.88 \times 10^{-5}\text{ cm.} = 5{,}880\text{Å} \qquad \lambda = \frac{1}{\Delta E'} = \frac{1}{16975} \quad \lambda = 5.88 \times 10^{-5}\text{ cm.} = 5{,}880\text{Å}$$

Wavelength calculations are simplified if energy in the equation $\Delta E = hc/\lambda$ is expressed not in ergs, but in units of ergs/hc. These turn out to have the dimensions of cm^{-1} and are called wave numbers, or reciprocal centimeters. Such energy figures are called E′. On the right side of figure one and in the calculations above, the energy levels of the sodium atom are measured in cm^{-1}. The advantage of expressing E′ in cm^{-1} is that the equation, written in terms of E′, becomes simply:

$$\frac{\Delta E}{hc} = \Delta E' = \frac{1}{\lambda} \qquad \text{and} \qquad \lambda = \frac{1}{\Delta E'}$$

To find the wavelength in reciprocal centimeters for an energy level transition, take the reciprocal of $\Delta E'$. The calculation of λ by this approach is shown above. Notice that it gives the same result as that obtained by measuring ergs, but the calculations required are easier.

Hydrogen Energy Levels

The simplest atomic spectrum is that of the hydrogen atom. Niels Bohr, in 1913, theoretically explained atomic spectra with a hydrogen atom model. According to Bohr's theory, the energies E'_n detectable in a hydrogen atom are all representable by the following equation:

$$E'_n = \frac{-R}{n^2}$$

where R is a constant predicted by the theory and n is an integer, 1, 2, 3, ... called a quantum number. All the lines in the hydrogen atom spectrum are associated with specific energy levels in the atom and are accurately predictable by Bohr's equation.

The value of R in the above equation was found to be 109,737.31 cm.^{-1}, one of the most accurate of all physical constants. On this basis, the above equation takes the form:

$$E'_n = \frac{-109{,}737.31\text{ cm}^{-1}}{n^2}$$

There are several ways to analyze an atomic spectrum, given the energy levels of the atom. One simple method is to calculate wavelengths theoretically using Bohr's equation and then matching these numbers against those derived from spectrophotometer readings.

Procedure

In this exercise you will calculate energy levels of hydrogen using Bohr's equation, calculate wavelengths of hydrogen spectrum lines using reciprocal centimeters, and calculate ΔE in both reciprocal centimeters and angstroms. You will then compare your calculations against the wavelengths of some lines in the hydrogen atomic spectrum derived from spectrophotometer readings which will be furnished.

Steps

A. Energy level calculations: given the expression for E'_n in Bohr's equation, it is possible to calculate energy levels in cm.^{-1} for the hydrogen atom.

1. Calculate energy levels in cm.^{-1} for each of the ten lowest levels of the hydrogen atom, starting with $n = 1$ as the first quantum number. Remember that the energies are all negative and that the lowest energy level should have the largest negative value. Enter these values in the following table, figure two.

The ten lowest energy levels of hydrogen

quantum number	energy E'_n in cm.^{-1}	quantum number	energy E'_n in cm.^{-1}

Figure 2

2. On the following graph, figure three, plot on the y axis each of the six lowest energies. The y axis of the graph is to have zero cm^{-1} at the top of the graph and 100,000 cm^{-1} at the bottom. Label the y axis in increments of 10,000 cm^{-1}. After spotting the values for the six lowest energies on the y axis, draw a horizontal line at each energy level and write the energy values on the lines.

B. Calculation of wavelengths of hydrogen spectrum lines: hydrogen spectrum lines all arise from jumps made by the atom from one energy level to another. The wavelength in centimeters of these lines can be calculated by applying Planck's law. $\Delta E'$ in the following equation is the difference in energy between any two levels.

$$\Delta E' = En'_{hi} - En'_{lo} = \frac{1\ \text{cm}^{-1}}{\lambda}.$$

where En' high (hi) and low (lo) represent different energy states of the atom. Rearranging the equation yields

$$\lambda\,(\text{cm}) = \frac{1}{\Delta E'}$$

Solving for angstroms is done as follows: $\text{Å} = \lambda\,(\text{cm.}) \times 1 \times 10^{8}$

Figure 3

3. The chart below, figure four, is structured so that two entries can be made at the intersection of lines running between two quantum numbers whose energy values are to be taken from the table created in question one above. The energy value for point 4 in both the vertical and horizontal columns is the same. Calculate and write $\Delta E'$ in the upper half of each box. In the lower half of the box, solve for and write the wavelength in angstroms associated with that value of $\Delta E'$.

Wavelength calculations in the hydrogen spectrum

n_{higher} / n_{lower}	6	5	4	3	2	1
1						
2						
3						
4						
5						

Figure 4

C. Matching calculated and observed lines in the hydrogen spectrum: compare the wavelengths calculated in angstroms in figure four with those derived from spectrophotometer readings listed in figure five below. If the calculations were properly done, the wavelengths should match several **(not all)** of those which were observed with the spectrophotometer.

4. In the box opposite each wavelength in figure five below, enter quantum numbers for both the upper and lower states whose energy differences yield those wavelengths that can be matched. This is to be done by comparing the above calculated angstrom values with the values given below which were derived in angstroms from spectrophotometer readings.

Some wavelengths (in Å) in the spectrum of hydrogen

$n_{hi} \rightarrow n_{lo}$	$n_{hi} \rightarrow n_{lo}$	$n_{hi} \rightarrow n_{lo}$
972.5:	4101.7:	10,049.8:
1025.7:	4300.5:	10,938:
1215.7:	4861.3:	12,808:
3889.1:	6562.8:	18,751:
3970.1:	9546.2:	40,500:

Figure 5

5. Some of the observed wavelengths in figure five, the hydrogen wavelength chart, can not be matched against the calculated wavelengths in question three. It is possible to deduce those quanta states which yield the observed wavelengths. This is done by examining the transitions that have been marked on the graph in question two and by examining the quantum state pairings marked on the wavelength chart in question four above. Deduce the quantum numbers which would yield the unmatched wavelengths above. Write the deduced quantum numbers in figure six below.

Unassigned wavelengths

wavelength observed	probable transition $n_{hi} \longrightarrow n_{lo}$	E transition	calculate in cm^{-1}

Figure 6

D. The Balmer series: in 1886 Balmer showed that the lines observed up to that time in the spectrum of the hydrogen atom had wavelengths expressable by a simple equation. He was limited experimentally to wavelengths in the visible and near ultraviolet regions, from 2,500 Å to 7,000 Å. The lines in this range are referred to as the Balmer series.

6. Study the entries on the chart in question three and determine the primary characteristic of the lines in the Balmer series.

7. What would be the longest possible wavelength for a line in the Balmer series, ______________ Å, and the shortest, ______________ Å?

8. Why must all lines in the hydrogen spectrum between 2,500 Å and 7,000 Å belong to the Balmer series?

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

- angstrom
- Balmer series
- Bohr's theory
- erg
- excited state
- ground state
- ionization
- Planck's law
- quantum
- quantum theory
- reciprocal centimeter

Review

6. The Atomic Spectrum of Hydrogen

Name __________________________

Date __________________________

Short answer

1. Given $\lambda = \frac{1}{\Delta E'}$ solve for the change in energy of a wavelength of 5,800 Å in cm^{-1}.

   $\Delta E'$ = __________________

2. An unknown atom has an ionization energy of 5,000 cm^{-1}. Given the following conversion factors, find the ionization energy of this unknown atom in ergs, joules, and kcal/mole.

   $$1 \text{ erg} = 1 \times 10^{-7} \text{ joules} = 5.0 \times 10^{15} \text{ cm}^{-1} = 1.439 \times 10^{11} \text{ kcal/mole}$$

   ____________________ ergs ____________________ joules ____________________ kcal/mole

3. The lowest energy level of the lithium atom lies 43,487 cm^{-1} below the energy required to ionize the atom. The first excited state of lithium is at 28,579 cm^{-1} below ionization energy. What wavelength of light is emitted when a lithium atom makes a transition from the first excited state to the ground state?

   ____________________ cm^{-1}

4. What is the ionization energy of lithium: ________________ in cm^{-1}, ________________ angstroms,

   ________________ joules, and ________________ in kcal/mole?

7. Laboratory

Enzyme Activity

When you have completed this laboratory you should be able to:

1. Use the spectrophotometer to measure biological activity.
2. Name the substrate and products of a peroxidase catalyzed reaction.
3. Explain the role of guaiacol in the enzymatic reactions occurring in this laboratory.
4. Describe how temperature, pH, enzyme concentration, and substrate concentration affect reaction rate.
5. Explain why peroxidase is an essential enzyme for all aerobic, or oxygen utilizing cells.

Purpose

Enzymes are biological catalysts which facilitate the thousands of chemical reactions that occur in living cells. All living things contain enzymes. They are generally large proteins composed of several hundred amino acids. In an enzyme catalyzed reaction, the substance to be acted on, or substrate, binds to an active site on the enzyme. The enzyme and substrate are held together in an enzyme-substrate complex by hydrophobic interactions, hydrogen bonds, and ionic bonds. The enzyme then converts the substrate to reaction products in a process that often requires several chemical steps. The products are then released into solution, and the enzyme is free to form another enzyme-substrate complex. Enzymes are used in reactions but not used up by them. One enzyme molecule can carry out thousands of reaction cycles every minute.

Each enzyme is specific to a certain reaction because it is unique in both its amino acid sequence and its three dimensional structure. The active site also has a specific shape so that only one, or a few, of the thousands of components present in the cell can interact with it. Any substance that blocks or changes the shape of the active site on an enzyme will interfere with the enzyme's effectiveness. If these changes are large enough, the enzyme ceases to act and is said to be denatured.

The purpose of this laboratory is to analyze the reactions of the enzyme peroxidase under varying physical conditions. The spectrophotometer will be used to provide data on these reactions.

Pre-lab

Materials needed for testing the enzymatic activity of peroxidase:

Equipment

blender
beakers
funnels
spectrophotometer
cuvettes
test tubes
test tube holders
watch or stopwatch

test tube rack
scissors
marking pencils
1 ml. graduated pipets
10 ml. graduated pipets
boiling water baths at approximately
4, 15, 30, and 37° C.

Materials

- rutabaga, turnip, or radish
- guaiacol solution
- 3% hydrogen peroxide solution
- tissues
- filter paper
- buffer, pH 3
- buffer, pH 7
- buffer, pH 11
- 10% hydroxylamine HCl
- tape
- parafilm
- food coloring

Special Preparations

1) Test the hydrogen peroxide to assure that it has not degraded.

2) For the guaiacol solution, mix 0.22 ml. of guaiacol with 100 ml. distilled water. Store in brown glass or aluminum foil covered bottles for no longer than two days prior to the laboratory.

3) For the 10% hydroxylamine HCl, mix 1 ml. hydroxylamine HCl with 9 ml. distilled water.

4) Since peroxidase activity varies with the age and condition of the root, prepare the extract and test its activity before the laboratory period. Adjust the amount of root and ml. of extract used in the laboratory as required. Students may wish to perform this part of the pre-lab as a way to familiarize themselves with laboratory materials and equipment.

a) Peel and cut the root vegetable into approximately 1 gram cubes.

b) Place one gram of the root vegetable in a blender with 100 ml. distilled water or pH 7 buffer.

c) Blend it thoroughly at high speed for about 1 minute.

d) Filter the extract.

Time Required

This enzyme activity laboratory requires four classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students require the following: spectrophotometer, stopwatch, cuvettes, test tubes, test tube holders, rack, marking pencil, pipets, distilled water, root extract, guaiacol solution, and hydrogen peroxide.

Peroxidase

In this laboratory you will study the enzyme peroxidase from a root vegetable. Peroxidases are widely distributed in plant and animal cells and catalyze the oxidation of organic compounds by hydrogen peroxide as follows:

$$RH + 2H_2O_2 \xrightarrow{\text{peroxidase}} ROH + H_2O$$

1. What are the substrates of this reaction?

2. What are the products?

Any cell using molecular oxygen in its metabolism will produce small amounts of H_2O_2 as a highly toxic by-product. It is critical that the H_2O_2 be quickly removed by enzymes such as peroxidase before damage is done to the cell.

You will use a reducing agent, guaiacol, that changes color when it is oxidized. This change can be easily measured with the spectrophotometer.

$$\underset{\text{(colorless)}}{4\ \text{guaiacol}} + 2H_2O_2 \xrightarrow{\text{peroxidase}} \underset{\text{(brown)}}{\text{tetraguaiacol}} + 8H_2O$$

3. How is this similar to the reaction in some fruits and vegetables when their flesh is cut and exposed to air?

Absorbance

A colored solution such as oxidized guaiacol solution appears that way because some of the light entering the solution is absorbed by the colored substance. A clear solution will allow almost all of the light to pass through. The amount of absorbance can be determined by using a spectrophotometer, which measures quantitatively what fraction of the light is transmitted through a given solution. It also indicates on the absorbance scale the amount of light absorbed compared to that absorbed by a clear solution. The darker the solution, the greater the absorbance.

Transmittance (T) is the ratio of the transmitted light (I) of the sample to the incident light (I_0) on the sample.

$$T = \frac{I}{I_0}$$

This value is multiplied by 100 to derive the term % T. For example:

$$T = \frac{50}{100} = 0.50 \qquad \text{and} \qquad \%T = \frac{50}{100} \times 100 = 50\%$$

Absorbance (A) is the logarithm to the base 10 of the reciprocal of the transmittance:

$$A = \log_{10} \frac{1}{T}$$

For example, if %T of 50 was recorded then $A = \log_{10} \frac{1}{0.5} = \log_{10} 2.0$.

Thus, $\log_{10} 2.0 = 0.602$ A. One advantage of absorbance calculations is that they are logarithmically rather than arithmetically derived, which allows the use of Beer's law. Beer's law states that over a given concentration range the concentration of solute molecules is directly proportional to absorbance. Beer's law can be expressed as:

$$\log_{10} \frac{I_0}{I} = Ecl = A;$$

where,

I_0 = intensity of incident light;
I = intensity of transmitted light;
E = the molar extinction coefficient, a constant value that is characteristic for each substance;
c = concentration of solution; and,
l = the length of the light path through the solution in centimeters.

The usefulness of absorbance can be seen in figure one:

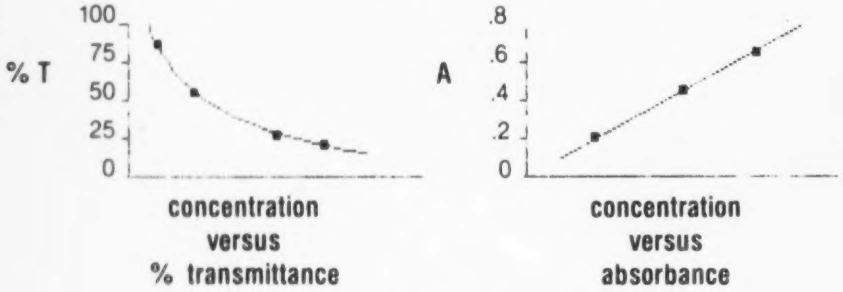


Figure 1

Because absorbance is directly proportional to concentration, a plot of absorbance versus concentration yields a straight line, the slope of which represents the constant E. Such a plot is called a calibration, or standard curve. Once several points have been plotted, the student can extrapolate the intervening points by connecting the known points with a straight line.

4. What would be the advantages of working with a straight line graph in analyses of chemical reactions?

____

____

Beer's Law tends to break down in excessively strong concentrations. If the concentration/absorption figures being plotted begin to curve, it is necessary to dilute the unknown sample by some factor until the absorbance readings intersect with the straight line part of the graph where concentration is proportional to absorbance. It is then possible to determine the unknown concentration and multiply the value by the dilution factor.

Steps

A. When you are ready to practice using the spectrophotometer, use figure two to follow standard spectrophotometer procedures.

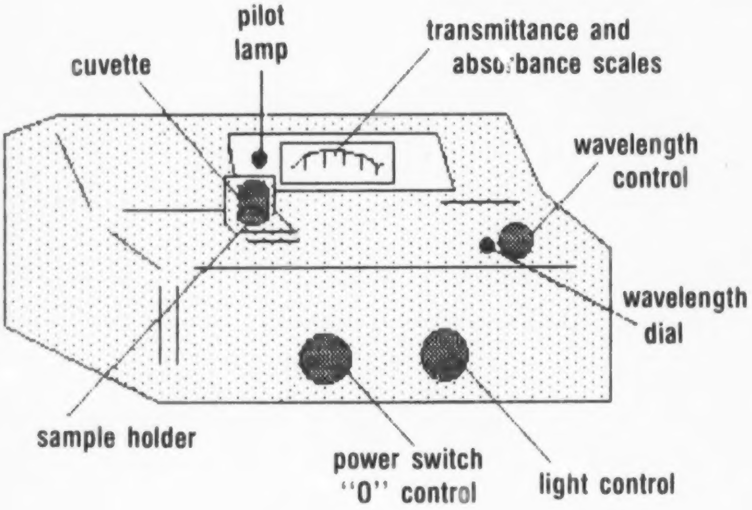

pilot lamp
cuvette
transmittance and absorbance scales
wavelength control
wavelength dial
sample holder
power switch "0" control
light control

Figure 2

1). Turn on the instrument with the power switch knob. Allow 5 minutes for warm up.

2). Adjust the wavelength dial to 500 nanometers.

3). With the sample chamber empty and the cover closed, use the power switch knob to set the meter needle to read infinity absorbance.

4). Fill a spectrophotometer tube or cuvette halfway with distilled water to make a blank. Wipe it free of moisture or fingerprints with a lint free tissue, and insert it into the sample holder. Line up the etched mark with the raised line on the front of the sample holder, and close the cover.

5). With the right hand knob, adjust the meter to read zero absorbance.

6). Remove the cuvette, empty it, and shake it as dry as possible. This precaution is essential for accuracy.

7). Fill a sample cuvette with food coloring tinted water and insert in the spectrophotometer.

8). Read absorbance directly from the meter. Rinse the cuvette with clean water and shake it as dry as possible.

9). It is necessary to reset the machine to infinity absorbance and zero absorbance with a blank before each set of readings because the settings can drift. Whenever the wavelength is changed, the infinity and zero absorbance also must be reset.

B. Once you have learned how to operate the spectrophotometer, the next preparatory step in the study of the kinetics of the peroxidase reacton will be to learn how to pipet. Pipets will be used to measure the solutions. Practice using a pipet with sterile water until you are comfortable with the techniques of pipetting.

C. Label the pipets with a marking pencil so each one can be reused with the proper solution. Be sure to use a different pipet for each solution so that the reagents are not ruined by cross contamination.

D. Label a cuvette #1. It will contain everything but the final compound needed for a reaction to occur, and it will be the blank for the experiment. Add by pipet 0.4 ml. guaiacol, 3.0 ml. of root extract, and 6.6 ml. distilled water. Mix well.

E. Label two test tubes #2 and #3. The contents of #2 and #3 will be mixed to start the reaction.

1). Tube #2: add by pipet 0.4 ml. guaiacol, 0.6 ml. 3% H_2O_2, and 3.0 ml. distilled water.

2). Tube #3: add by pipet 3.0 ml. root extract and 3.0 ml. distilled water.

F. Adjust the spectrophotometer to zero absorbance using cuvette #1, following steps specified in A above. You have now set the instrument so that any difference in the meter reading with a change in the sample will reflect a difference in oxidized guaiacol concentration.

5. Why is this step important? ____

____

____

G. Obtain a stopwatch, or use a watch with a second hand or timer device on it, to time step H.

H. Obtain a clean cuvette and label it #4. You will have **only** 20 seconds to perform the following steps of mixing, pouring, wiping, inserting, and reading the spectrophotometer. When you are ready, do the following:

1). Mix the contents of test tubes #2 and #3, pour the contents back and forth two times quickly, and then pour into cuvette #4. Start the stopwatch or timer when the tubes are first mixed. (t = 0 when the tubes are first mixed.)

2). Wipe the outside of the cuvette and place it in the spectrophotometer.

3). Take your first reading 20 seconds after the tubes were mixed. (t = 20 seconds.)

4). Continue to read the absorbance every 20 seconds for 4 minutes.

I. Repeat the procedure twice more to verify the accuracy of your base line data.

6. Record the readings in figure three.

time (seconds)	run 1 (base line)	run 2	run 3
20			
40			
60			
80			
100			
120			
140			
160			
180			
200			
220			
240			

Figure 3

7. Graph the absorbance versus time in your base line run in figure four.

Figure 4

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The preceding graph, figure four, represents the base line against which enzyme activities during following laboratory periods will be compared. During subsequent procedures you will vary one condition at a time and compare the results against the base line. Since your base line will affect all subsequent readings, have your instructor check the base line graph before proceeding.

Procedure: Hour 2

Students need the same supplies for hour 2 as they used for hour 1.

It is possible to determine an enzyme's optimum, or most efficient reaction rate by testing varying physical conditions of reaction and by determining which factors contribute to a rapid and complete enzymatic reaction. Since enzymes are organic compounds, it is to be expected that their optimum reaction rates are bounded by the narrow and precise conditions found within living systems. Hours 2, 3 and 4 of this laboratory will be devoted to testing the physical conditions which provide levels of optimum activity for peroxidase.

It has been found that the following conditions impact upon the activity of enzymes.

Concentration of Enzyme and Substrate

By varying the amounts of the enzyme and/or the substrate it works upon, the speed of the reaction is either speeded up or slowed down. The reaction will only proceed as rapidly as there are materials available to react, and even though enzymes are not used up in the reaction, they are required for it to work.

Temperature

Chemical reactions speed up as temperature is raised. As temperature increases, more molecules acquire enough kinetic energy to begin reacting. However, if the temperature of an enzyme catalyzed reaction is raised high enough, a temperature optimum is reached. Above this temperature optimum, kinetic energy is so great that the structure of the enzyme starts to degrade, leading to denaturation. Many proteins and enzymes are denatured by temperatures around 40° to 50° C., but a few can even survive boiling. It depends on the particular enzyme and the type of living system to which it is adapted.

pH

pH is given on a logarithmic scale which measures the acidity, or hydrogen ion concentration in a solution. The scale runs from 0 to 14, with 0 being the highest in acidity and 14 the lowest, or most basic. A pH of 7 is neutral. Amino acid side chains contain groups such as $-COOH$ and $-NH_2$ which readily gain or lose hydrogen ions. As pH is lowered, an enzyme will tend to gain hydrogen ions, and eventually enough side chains will be affected to disrupt the active shape of the enzyme. The opposite happens as the pH is raised, but with similar negative results. Many enzymes have an optimum in the neutral pH range and are denatured by low or high pH. Some enzymes, such as those which act in the acidic human stomach, have an appropriately low pH optimum.

Salt Concentration

If salt concentration is very low or very high, the charged amino acid side chains of enzymes stick together. The enzymes will be denatured and form an inactive precipitate from the solution. An intermediate salt concentration, such as that of blood (0.9%), is the optimum for most enzymes.

Activators and Inhibitors

Molecules other than the substrate may interact with enzymes. If the molecule increases the rate of the reaction, it is called an activator. If it decreases the rate of reaction, it is called an inhibitor. The cell uses such molecules to regulate reaction rates. Any substance that tends to unfold the enzyme, such as an organic solvent or detergent, will act as an inhibitor. Some inhibitors act by reducing disulfide bridges that stabilize the enzyme's structure. Many inhibitors react with side chains in or near the active site. Others may damage or remove the active site on the enzyme. Many well known poisons are enzyme inhibitors which interfere with the active sites on critical enzymes.

Steps

A. The effect of enzyme concentration will now be measured by doubling the amount of enzyme used to establish the base line during hour one.

8. Notice that during this laboratory cuvette #1 always contains 10 ml., and test tubes #2 and #3 together always total 10 ml. Why is this important?

B. Follow the procedure as outlined in hour 1, but vary the materials in the cuvettes in this fashion:

1). Cuvette #1. Add 0.4 ml. guaiacol, 6.0 ml. root extract, and 3.6 ml. distilled water.

2). Test tube #2. Add 0.4 ml. guaiacol, 0.6 ml. 3% hydrogen peroxide, and 2.0 ml. distilled water.

3). Test tube #3. Add 6.0 ml. root extract and 1.0 ml. distilled water.

9. Repeat the procedure for reading and recording data, using figure five.

time seconds	run
20	
40	
60	
80	
100	
120	
140	
160	
180	
200	
220	
240	

Figure 5

C. Next, half the amount of enzyme will be used as that which was used in determining the base line during hour one. Follow the procedure as outlined in hour one, but vary the materials as follows:

1). Cuvette #1. Add 0.2 ml. guaiacol, 1.5 ml. root extract, and 8.3 ml. distilled water.

2). Test tube #2. Add 0.2 ml. guaiacol, 1.5 ml. 3% hydrogen peroxide, and 3.8 ml. distilled water.

3). Test tube #3. Add 1.5 ml. root extract and 3.0 ml. distilled water.

10. Repeat the procedure for reading and recording the data by using figure six.

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 6

11. How does changing the concentration of enzyme affect the rate of the reaction?

12. In figure eight, graph the original base line data. Then graph absorbance versus time from data charted after both doubling and halving the concentration of peroxidase. The graph will be easier to read if the three lines are in different colors. Before graphing the results, record your color key in the figure seven chart.

Key to graph:

color	enzyme concentration

Figure 7

Figure 8

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D. Next you will experiment with the effect of substrate concentration by varying concentrations of the substrate, hydrogen peroxide.

1). Double the amount of hydrogen peroxide and run the test:

13. Cuvette #1. Add

Test tube #2. Add

Test tube #3. Add

14. Chart the results in figure nine.

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 9

2). Halve the amount of substrate, hydrogen peroxide and run the test:

15. Cuvette #1. Add

Test tube #2. Add

Test tube #3. Add

16. Chart the results in figure ten.

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 10

17. How does changing the amount of substrate affect the rate of reaction?

18. Plot absorbance versus time on the graph, figure twelve. Again, the graph will be easier to read if the three sets of data are in different colors. Plot the original base line data, then the data collected on the charts, figures nine and ten, while both halving and doubling substrate concentration.

Key to graph:

color	substrate concentration

Figure 11

Figure 12

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19. What conclusions can be reached concerning the effects of peroxidase, and hydrogen peroxide concentrations on reaction rates?

Procedure: Hour 3

Students require all materials and equipment used in laboratory hours 1 and 2, plus the boiling water baths. The water baths of 4°, 15°, 30°, and 37° C. should be set up before class.

The purpose of this exercise is to determine the effects of temperature on enzyme activity. The experiment will show the differences in peroxidase activity between 4 and 37 degrees centigrade. Use the same conditions as in the base line experiment, but run the reaction in the different temperature water baths. There will be some small percentage of error as the solutions change temperature after removal from the water baths and insertion in the spectrophotometer; however, this error does not skewer the results substantially. Record the exact temperature of each run.

A. Run the test on peroxidase activity at 4° C.

20. Record results in figure thirteen.

Water bath temperature = ____________________

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 13

B. Run the test on peroxidase activity at 15° C.

21. Record results in figure fourteen.

Water bath temperature = ____________________

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 14

C. Run the test on peroxidase activity at 30° C.

22. Record results in figure fifteen.

Water bath temperature = ____________________

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 15

D. Run the test on peroxidase activity at 37° C.

23. Record results in figure sixteen.

Water bath temperature = ________________

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 16

24. At what temperature did maximal activity occur? ________________

25. Plot results on the figure eighteen graph. For greater ease in reading the graph, use different colors for the different temperature runs. Include on the graph the original base line run. Its temperature should be room temperature. Use the figure seventeen key for the graph.

Key to graph:

color	temperature

Figure 17

Figure 18

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Procedure: Hour 4

Students will need the same materials for hour 4 as used during hour 1, with the addition of the buffers, pH 3, 7 and 11, and the 10% hydroxylamine HCl.

The purpose of hour four is to measure the effect of pH on enzymatic reaction rates. Use the conditions of the base line reaction, but replace the distilled water with the buffered solutions in each test. Record and chart each run.

A. Run the test on peroxidase activity at pH 3.

26. Record results in figure nineteen chart.

pH = ____________________

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 19

B. Run the test on peroxidase activity at pH 7.

27. Record results in the figure twenty chart.

pH = ____________________

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 20

C. Run the test on peroxidase activity at pH 11.

28. Record results in the figure twenty one chart.

pH = ____________________

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 21

29. Measure the pH of the distilled water so that the base line and buffer results are comparable.

What is the pH of the distilled water? ______________

30. At which pH is the enzyme most effective? ______________

31. Why is this important in living systems? ____

____

____

____

32. Plot pH results in the figure twenty three graph. Use different colors for the different pH runs for greater clarity. Use the figure twenty two chart to color key the graph. Plot the original base line run with the pH of distilled water.

Key to graph:

pH	color
3	
distilled water =	
7	
11	

Figure 22

D. Hydroxylamine is a small molecule whose structure is similar enough to hydrogen peroxide that it can bind peroxidase. Hydroxylamine is an inhibitor of peroxidase, because it attaches to the iron atom in peroxidase, just as hydrogen peroxide does. It is simple to test its effect on peroxidase activity.

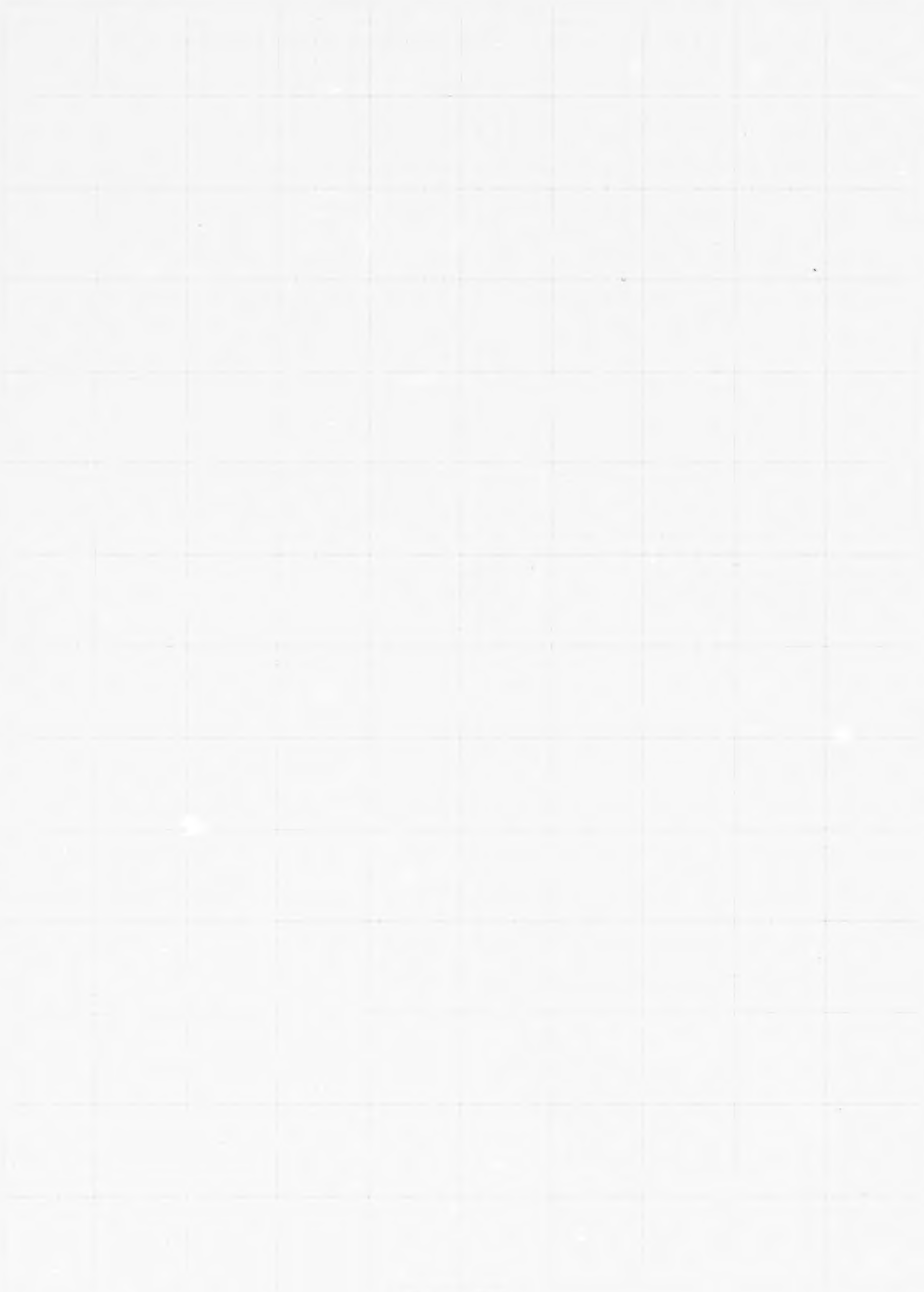

Figure 23

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Add 5 drops of 10% hydroxylamine to the root extract to be tested. Let the mixture stand for 1 minute. Repeat the base line reaction of hour one, but use the root extract with 10% hydroxylamine added.

33. Record results in the figure twenty four chart.

time seconds	run
20	
40	
60	
80	
100	
120	

time seconds	run
140	
160	
180	
200	
220	
240	

Figure 24

34. How does this compare with the original base line run? ______________________________

35. Is the peroxidase still active? ______________________________

Resources

Changeaux, Jean Pierre. "The Control of Biochemical Reactions". *Scientific American*, April 1965 (#1008).

Doolittle, Russell F. "Proteins". *Scientific American*, October 1985.

Koshland, D. E., Jr. "Protein Shape and Biological Control". *Scientific American*, October 1973 (#1280).

Mosbach, Klaus. "Enzymes Bind to Artificial Matrixes". *Scientific American*, March 1971 (#1216).

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

activator
active site
Beer's law
catalyst
denaturation
enzyme
guaiacol
inhibitor
peroxidase
pH
substrate

Review

7. Enzyme Activity

Name________________________________

Date________________________________

Multiple choice

______ 1. The pH of a substance represents the concentration of

a) H_2O
b) OH^-
c) H^+
d) G_2O_2

______ 2. Acids have pH ranges between

a) 1 - 6.9
b) 7
c) 7.1 - 14
d) 1 - 14

______ 3. Enzymes are part of a group of compounds called

a) carbohydrates
b) lipids
c) nucleic acids
d) proteins

______ 4. Chemicals which can attach themselves to enzymes and yet not react further with the enzyme, or other molecules, are called

a) activators
b) inhibitors
c) acids
d) bases

______ 5. Guaiacol is

a) a reducing agent
b) an inhibitor
c) an oxidizing agent
d) a binding agent

______ 6. Enzymes are

a) lipids
b) inhibitors
c) oxidizing agents
d) organic catalysts

______ 7. Peroxidase is an enzyme that changes

a) hydroxylamine to water
b) starches to sugar
c) hydrogen peroxide to water
d) colored solutions to clear

______ 8. Absorbance in the spectrophotometer is directly proportional to

a) concentration
b) the constant E
c) transmittance
d) dilution factors

______ 9. If salt concentration is very low or very high, the charged amino acid side chains

a) split apart
b) lose positive charges
c) become very active
d) stick together

______ 10. Optimum temperature in enzymatic activity refers to

a) temperature of maximum enzymatic activity
b) the higher the temperature the better
c) the only temperature at which the enzyme functions
d) the lower the temperature the better

Essay

What factors affect enzymatic activity, and why?

Answers Found: p 49 - #3 & 6; p 50 - #7; p 51 - #5; p 52 - #8; p 56 - #1, 2, 9, & 10; p 56 & 57 - #4; Essay: p 56 & 57.

8. Laboratory

Photosynthesis & Bioluminescence

When you have completed this laboratory you should be able to:

1. Use the spectrophotometer to measure the visible absorption spectrum of spinach chloroplasts.
2. Determine the wavelengths most useful for photosynthesis in spinach chloroplasts.
3. Understand the Hill reaction and its relationship to oxygen production and photosynthetic electron transport.
4. Demonstrate the effect of changing the level of light on electron transport in photosynthesis by measuring reduction of a dye in the Hill reaction.
5. Demonstrate how the stored energy of photosynthesis can be changed into light energy.

Purpose

Photosynthesis is the essential biochemical process of life. No life, neither that which produces its own food through photosynthesis nor that which ultimately derives its food from green plants, is possible without photosynthesis. It is a complicated chemical sequence which can best be expressed as:

$$nH_2O + nCO_2 \longrightarrow (CH_2O)_n + nO_2$$

The $(CH_2O)_n$ in the equation is most often glucose, $C_6H_{12}O_6$.

Photosynthesis is carried on by organisms which are capable of "trapping" light in special light receptor systems. In green plants these light receptor systems are chloroplasts, and they are required for the light reactions of photosynthesis, in which light energy is converted to chemical energy (ATP or NADPH) through an electron transport system. The ATP or NADPH feed into the assembly line of the dark reaction, or Calvin cycle, where enzymes carry out carbon fixation by converting CO_2 into glucose.

The dark reactions of photosynthesis are so named because they can occur in the absence of light. During the dark reactions, the energy stored in ATP or NADPH is used to convert CO_2 and H_2O to primarily glucose molecules and oxygen gas. All life forms incapable of photosynthesis are, in one way or another, dependent upon that glucose for food.

The purpose of this laboratory is to explore some of the properties and reactions of photosynthesis, and to show how the energy obtained from photosynthesis can be converted into either the chemical energy of food or the light energy of bioluminescence.

Pre-lab

Materials needed for exploring the properties and reactions of photosynthesis:

Equipment

spectrophotometer
cuvettes
test tube rack
centrifuge
centrifuge tubes
glass rod
microscope slides
coverslips
microscope
ice bucket
(or beakers)
small test tubes
50 ml. flask
Pasteur pipets
blender

Materials

fresh spinach leaves
5 M. cold buffered sucrose solution
80% acetone
cheesecloth
phosphate buffers (pH 6.5 & 7.8)
2,6-dichlorophenol-indolphenol dye
distilled water
aluminum foil
ice
firefly lanterns
ADP (adenosine 5′-diphosphate)
PMS (phenazine methosulfate)
diluted chlorophyll extract

Special Preparations

1) Spectrophotometers: instruments used by the students should be equipped with a wide range phototube and filter (CE A95) to measure the absorption spectrum of chlorophyll. Standard spectrophotometers can not be zeroed above 650 nanometers (or 669 in some cases) with the visible phototubes, and students will not be able to see the absorption maximum in the 660 to 670 nm. region. The wide range phototube can be used in demonstrating absorption spectra in the Hill reaction as well, if students wish to quantify this laboratory after hour 2.

2) 80% acetone: mix 200 ml. distilled water and 800 ml. acetone. Keep tightly covered.

3) 0.5 M. cold, buffered sucrose solution: dissolve 71.6 grams sucrose ($C_{12}H_{22}O_{12}$), 0.5 g. NaCl, and 2.72 g. KH_2PO_4 in 800 ml. distilled water. Adjust to pH 6.8 with 1 M. NaOH (10 g. NaOH dissolved in 25 ml. distilled water) and bring the total volume to 1 liter. Store tightly covered in a refrigerator.

4) Diluted chlorophyll extract: dry fresh spinach leaves as much as possible. Add 200 ml. acetone to a blender full of dried spinach and blend. Pour off the acetone into a flask and cover it. Re-extract the residue in the blender with 100 ml. 95% ethanol. Pour off the alcohol extract and combine it with the acetone extract. Filter the combined extracts through a Whatman #1 filter paper. If the extract is not a nice dark green color, add enough $CaCl_2$ to separate the extract into two layers. The pigments will become concentrated in the upper layer and can be removed with a pipet or isolated with a separatory funnel. This produces a concentrated pigment. Dilute with 80% acetone until the absorbance at 400 nm. is about 0.7 on the spectrophotometer. Keep dark and refrigerated in a tightly covered, foil-wrapped bottle.

Note: Students can spend class time making the chlorophyll extract as a way to familiarize themselves with the laboratory.

5) 2,6-dichlorophenol-indolphenol dye, or 2.5×10^{-4} M. 2,6-dichlorophenol-indolphenol, which is known as DCIP: dissolve 72.5 mg. DCIP in 1 liter distilled water. Store in foil-covered, tightly-stoppered bottles.

6) Firefly lanterns: each laboratory group needs 4 lanterns. Lanterns are naturally available in some areas. If it is the wrong time of year, or the wrong area, they may be purchased commercially, such as Sigma, #FFT.

7) ADP (adenosine 5′-diphosphate): available from a few suppliers, including Sigma, #A 2754.

8) PMS (phenazine methosulfate): available from a few suppliers, including Sigma, #P 9625.

Time Required

This photosynthesis laboratory requires three classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

The absorption spectrum of spinach chlorophyll will be studied during this hour.

Students need the following materials and equipment: spectrophotometer, cuvettes, 80% acetone, diluted chlorophyll extract, tissues.

A given molecule can occupy only particular energy levels, that is, it can absorb quanta of only certain specific energies (or wavelengths). An absorption spectrum is a plot for a given compound of the degree to which light is absorbed at given wavelengths. The fact that the peaks in an absorption spectrum are smoothly rounded, rather than sharp spikes, suggests that a given excited state is not a narrow band of energy; rather, it consists of substantial energy sublevels, differing by small increments. Therefore, absorption spectra are very good "fingerprints" of compounds, and unknown compounds sometimes can be identified by their absorption spectra.

Steps

Students should review spectrophotometer procedure as detailed in laboratory #7 on enzyme activity. Additionally, remember that the solvent, acetone, used in this experiment dissolves plastic. **Be careful not to spill any solvent on or in the spectrophotometer.**

A. Turn on the spectrophotometer and allow it to warm up for 5 - 10 minutes.

B. Fill a cuvette halfway with 80% acetone to use as a blank.

C. Fill a second cuvette halfway with diluted chlorophyll extract.

D. Turn the wavelength control knob to 400 nm. Zero the instrument by inserting the blank and adjusting the control knob to zero absorbancy. **Do not forget that the white line of the cuvette should face you.**

E. Insert the cuvette containing the chlorophyll extract, and take the first reading.

F. Change the wavelength to 410 nm., reset the spectrophotometer, and take the second reading.

G. Continue taking readings every 10 nm. until reaching 700 nm.

1. Record these readings in the chart, figure one, below.

wavelength	absorbance
400 nm	

wavelength	absorbance

Figure 1

Figure 2

2. Graph wavelength versus absorbance from figure one onto the preceding graph, figure two. Label each absorbance maximum (where the absorbance rises and then falls again) with its corresponding wavelength. If there are not at least two absorbance maxima in the spectrophotometer readings, go back to the regions of high absorbance and take more readings.

3. Does the absorption spectrum of dilute chlorophyll extract from spinach leaves indicate that spinach has more than one photosynthetic pigment in it? What might account for this?

4. Is there any wavelength at which no absorbance at all occurs? Why or why not?

Although the absorption spectrum graph relates light absorption to wavelength, the biological effectiveness of light can be plotted as a function of wavelength. The resulting graph is called an action spectrum. Action spectra graphs plot relative rate of photosynthesis against wavelength.

Optional Activities

Students may wish to trace photosynthetic abilities of a particular plant over a period of time. Each student should "adopt" a plant and track its chlorophyll absorption spectra over a period of two months, or longer, using the following procedures. When done, prepare a formal laboratory report to be handed in to your instructor.

1) Follow the procedures for hour 1 of this laboratory, except use the chosen plant's leaves for making the dilute chlorophyll extract.

2) Take spectrophotometer readings between 400 to 700 nm. on the chlorophyll extract. Collect the leaves fresh and test every two weeks during the period.

3) Record readings in a chart similar to the one in hour 1 of this laboratory, graph wavelength versus absorbance, and date each entry. These will document light absorbance by the plant over an extended period.

4) Compare findings with other students who "adopted" other plants. Consider similarities and differences in findings. What influences might such phenomena as environment, seasonal cycles, and species have on the data?

Procedure: Hour 2

The Hill reaction (the phase of photosynthesis which requires light) will be studied during this hour.

Students need the following materials and equipment: fresh spinach leaves, 0.5 M. cold buffered sucrose solution, blender, centrifuge and centrifuge tubes, cheesecloth, glass rod, microscope, slides and coverslips, tissues, 0.1 M. phosphate buffer (pH 6.5), 2,6 dichlorophenol-indolphenol, distilled water, test tubes, test tube rack, aluminum foil, and a beaker or ice bucket with iced water.

Robin Hill of Cambridge University was the first to observe that illumination of isolated chloroplasts resulted in the production of oxygen gas. This light-induced production of oxygen through photosynthetic electron transport is called the Hill reaction. The general reaction may be simplified thus:

$$H_2O + A \xrightarrow{\text{light}} AH_2 + 1/2\ O_2$$

in which A is a general hydrogen acceptor and AH_2 is the reduced form. Light provides energy to move electrons from water during the Hill reaction. This is the opposite of respiration in which electrons join with O_2 to form H_2O. $NADP^+$ is the electron carrier (A in the above reaction), and it is reduced to NADPH.

In this experiment the student will use an artificial electron acceptor, the dye 2,6 dichlorophenol-indolphenol, as the A in the above reaction. As the dye accepts electrons liberated by light energy, there should be a change in color, indicating that the dye has been reduced.

Steps

A. Prepare the chloroplast suspension as follows. This suspension will be used in both hours 2 and 3 of this laboratory.

Note: The following procedure must be carried out using cold solutions and glassware, as chloroplasts deteriorate at warm temperatures.

1). Take 50 grams of spinach leaves and remove their midribs. Homogenize the deveined leaves with 100 ml. 0.5 M. cold, buffered sucrose solution in a blender for 30 seconds.

2). Filter the resulting suspension through two layers of cheesecloth that have been chilled and moistened with the sucrose solution.

3). Centrifuge the filtrate at 50 G (low speed, or speed 3 in a clinical centrifuge) for 10 minutes to remove the debris.

4). Decant the supernatant to a fresh, chilled centrifuge tube and discard the pellet.

5). Recentrifuge the supernatant at 1000 G (higher speed, or speed 6 in a clinical centrifuge) for 8 minutes to pelletize the chloroplasts.

6). Decant the supernatant and discard.

7). Gently resuspend the pellet containing chloroplasts in 3 ml. cold, buffered 0.5 M. sucrose solution, using a clean, chilled glass rod. Treat the chloroplasts gently because they will be fragile after having been centrifuged.

B. Place a drop of chloroplast suspension on a clean, chilled, glass microscope slide. Add a coverslip, and observe with a microscope.

5. What shape and color are the chloroplasts?

If there are no chloroplasts, or they are in poor condition, the chloroplast suspension procedure will have to be redone. The second time be more careful with chilling and centrifuging.

Note: The following procedure must be carried out using cold solutions and glassware, as chloroplasts deteriorate at warm temperatures.

C. Add the following to each of two test tubes:

1). 2 ml. 0.1 M. phosphate buffer (pH 6.5).

2). 2 ml. 2,6 dichlorophenol-indolphenol dye.

3). 6 ml. distilled water.

D. Mix thoroughly. Add 4 drops chloroplast suspension to each tube. Mix thoroughly but gently.

E. Immediately wrap one test tube in aluminum foil, so that all light is excluded.

F. Place both test tubes in a beaker or bucket of iced water and leave them for 20 minutes.

G. Remove the test tubes from the iced water, and remove the foil from the one test tube. Observe.

6. Record observations in the chart, figure three, below.

condition	initial color	final color
light		
dark		

Figure 3

7. Which compound(s) is (are) reduced in this reaction?

8. Which compound(s) is (are) oxidized in this reaction?

9. The chemical structure of 2,6 dichlorophenol-indolphenol is shown below. At what point(s) does the compound accept electrons?

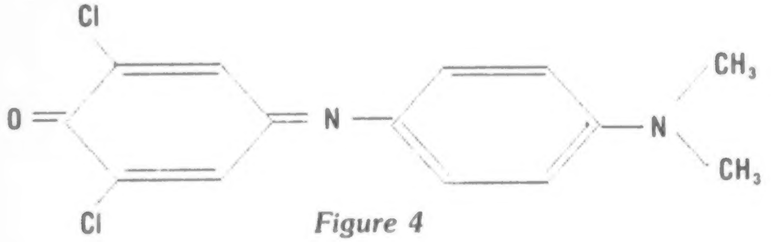

Figure 4

10. Is it possible that the rate of dye reduction might be tied to the intensity of light? If light intensity could be increased without heating the chloroplasts, would the rate of photosynthesis keep increasing indefinitely? Explain.

11. The fresh water plants *Elodea* and *Anacharis* are both commonly found in tropical fish stores. Both plants release oxygen gas as bubbles in the water in direct proportion to their photosynthetic activity. How might you devise an experiment using one of these plants to measure photosynthetic rate and also maximum wavelength absorbance? How might you include an action spectrum of the photosynthetic pigments of the plant in the experiment? Answer in the space provided below.

Procedure: Hour 3

Bioluminescence will be studied during this hour.

Background

A classic story about bioluminescence occurred during WWII. American soldiers fighting in the Philippine jungles were mystified as to how Japanese attackers could strike with such coordinated effectiveness on dark nights. After the war the Americans learned that the Japanese had netted small phosphorescent fish, dried them, and ground the dried fish into a powder which was carried in leather pouches on their belts. On even the darkest nights the Japanese could communicate between detachments by using hand signals naturally illuminated with this fish powder.

During the Arab Israeli War of 1967, a midnight patrol of Israeli soldiers spotted a faint green light hovering in the waters of the Red Sea along the shores of the Sinai Peninsula. They fired explosive shells into the glowing area which they took to be enemy frogmen. Instead they killed a school of flashlight fish. These fish inhabit underwater caves and come to the surface of the sea on dark, moonless nights to forage for small organisms. Their sources of light are bioluminescent bacteria located in packets just under their eyes, which give off a steady green glow. The bacteria serve as efficient "headlights," and the flashlight fish can turn them on and off by blinking.

There are many other examples of bioluminescence in nature, but the majority of organisms with the trait are bacteria. Bioluminescent bacteria live both in the water and on the land. Wax moth larvae can glow in the dark because of bioluminescent bacteria living inside them. Bioluminescent bacteria, particularily the ones living in association with flashlight fish, live in pure cultures. Scientists have been able to use these pure culture bacteria in genetic recombination studies by employing them as markers.

Even stranger than glowing bacteria are bacteria cultures which can generate sufficient electricity to power a light bulb. These bacteria have a purplish red pigment in their cells which efficiently converts ATP into electrical energy. Research continues on turning these bacteria into living solar batteries.

Laboratory

Students need the following: the chloroplast suspension prepared and used in hour 2 of this laboratory, phosphate buffer (pH 7.8), firefly lanterns, centrifuge and centrifuge tubes, ice bucket or beaker with iced water, ADP, PMS, test tubes and rack, pipets, 50 ml. chilled flask, source of light (sunlight or lamp), and a darkened area.

The Dark Reaction

Plants use sunlight as a source of energy to produce ATP. ATP is stored in the stroma of chloroplasts, and it provides the energy for the synthesis of carbohydrates. This synthesis is called the dark reaction of photosynthesis. The dark reaction occurs whether or not light is available. It is a complicated chemical sequence which can best be expressed as:

$$AH_2 \text{ (from the Hill reaction)} + CO_2 \xrightarrow{ATP} \text{carbohydrates}$$

This reaction is reversed when animals ingest plants or other animals and then metabolically break down carbohydrates to yield ATP. ATP fuels their metabolic processes. Phrased differently, sunlight is the ultimate energy source, but ATP is the usable energy source. And ATP can be converted into food, light, or electricity.

Bioluminescence in Fireflies

In the following experiment, you will illuminate isolated chloroplasts to make ATP and then use the ATP as an energy source to cause firefly lanterns to "glow."

ATP provides the energy needed to attach a molecule of oxygen to a luciferin molecule in the firefly. Luciferin is the active molecule manufactured in firefly bodies which is responsible for bioluminescence. The enzyme luciferase breaks down the luciferin/oxygen complex. Light is emitted in the process (one photon per molecule). The energy of the end products of the reaction (CO_2 and AMP, among others) is less than that of the starting materials (luciferin-O_2 and ATP), and the energy difference is released as light. It is best expressed as:

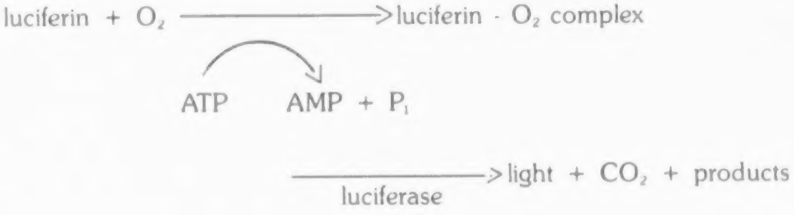

Figure 5

Steps

While preparing the chloroplast suspension for the hour 2 experiment, electron acceptor molecules were washed away. During this hour you will provide an artificial electron acceptor, phenazine methosulfate (PMS) to replace them. You also will add ADP and inorganic phosphorus (in the form of a phosphate buffer) because these also were washed out of the chloroplast suspension.

Note: Make sure equipment is kept ice cold throughout the procedure. Heat destroys isolated chloroplasts.

A. Put 12 drops phosphate buffer (pH 7.8) in a small test tube. Add 4 firefly lanterns. Homogenize the mixture with a chilled glass rod. Place test tube in iced water for 30 minutes.

B. While waiting, prepare the following chloroplast solution. In a chilled 50 ml. flask, using chilled pipets, carefully transfer 9 drops phosphate buffer (pH 7.8), 6 drops ADP, 6 drops PMS, and 6 drops chloroplast suspension (use the chloroplast suspension prepared during hour 2).

C. Gently shake the flask to mix it, and place the flask in an ice bucket or beaker with iced water. The container is to be illuminated for at least 10 minutes by either bright sunlight or lamps.

D. Suck the contents of each flask into separate chilled pipets (the firefly and chloroplast mixtures — at this point it is preferable to have a good pipetting technique). Place both pipets in separate test tubes. Squirt the firefly extract into its test tube and remove the pipet. Leave the chloroplast mixture in its pipet.

E. The last step must be done in the dark. Darken the laboratory (or visit a darkroom). Wait until your eyes become adjusted to the dark. Then squirt the chloroplast contents into the test tube containing firefly extract.

12. What do you see? ______________________________

____

____

____

13. How is ATP used in bioluminescence?

14. Outline the energy transfers from sunlight to the final demonstration of bioluminescence.

Resources

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Stoeckenius, Walther. "The Purple Membrane of Salt-Loving Bacteria," *Scientific American*, June 1976 (#1340).

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Terminology

Students should understand the following terms and concepts prior to taking the unit review.

action spectrum
ATP
bioluminescence
dark reaction
Hill reaction
luciferase
luciferin
photosynthesis

Review

8. Photosynthesis & Bioluminescence

Name ______________________________

Date ______________________________

Multiple choice

______ 1. The principal waste product of photosynthesis is

a) H_2
b) light
c) CO_2
d) O_2

______ 2. Chlorophyll appears green in color because the pigment

a) absorbs wavelengths of green light
b) reflects the color of surrounding pigments
c) absorbs blue and red wavelengths of light
d) absorbs all the wavelengths of light

______ 3. The rate of photosynthesis in a plant can be increased by increasing the

a) amount of CO_2
b) amount of O_2
c) amount of ATP
d) amount of ADP

______ 4. A graph which shows biological effectiveness as a function of wavelength is called a(an)

a) atomic spectrum
b) molecular spectrum
c) absorption spectrum
d) action spectrum

______ 5. The part of photosynthesis that requires light is called the

a) Calvin cycle
b) dark reaction
c) Hill reaction
d) Bohr reaction

______ 6. How many CO_2 molecules are needed to produce one glucose molecule?

a) 3
b) 4
c) 5
d) 6

______ 7. Chlorophyll can be changed from a ground state to an excited state by

a) oxidizing another molecule
b) reducing another molecule
c) absorption of a photon of light
d) fluorescence of a photon of light

______ 8. An end product of the dark reaction of photosynthesis is

a) ATP
b) NADPH
c) O_2
d) CO_2

Multiple choice

______ 9. Luciferin is an active molecule manufactured in the bodies of

a) flashlight fish
b) fireflies
c) bioluminescent bacteria
d) wax moths

______ 10. The ultimate energy source for life on earth is

a) the Hill reaction
b) sunlight
c) ATP
d) the dark reaction

Essay

1. Differentiate between an absorption spectrum and an action spectrum.

2. Summarize the events of photosynthesis, showing how the different pathways involved interlock to bring about the reduction of carbon dioxide.

Answers Found: p 73 - #1, 6, & 10; p 75 - #2; p 77 - #4; p 78 - #5 & 7; p 80 - #3; p 81 - #8 & 9; Essay 1: p 77; Essay 2: entire lab.

9. Laboratory

Respiration & Fermentation

When you have completed this laboratory you should be able to:

1. Determine the relationship between food concentration and energy production in yeast.
2. Distinguish between aerobic and anaerobic fermentation.
3. Demonstrate the procedure of serial dilution.

As discussed in the previous laboratory, ATP is the primary energy carrier in living systems, and sunlight is the ultimate energy source. Photosynthetic processes convert the sun's energy into chemical energy, most often stored temporarily in the high energy bonds of ATP. For long term storage, plants convert the ATP into carbohydrates. Non-photosynthetic organisms, as well as plants, are able to break down these carbohydrates and to convert them back to ATP. Common to all organisms is the process of glycolysis, which is the procedure of breaking down the simplest carbohydrate of all, glucose. Glycolysis occurs in the cytoplasm of the cell. We will consider this process in the following laboratory.

Respiratory Pathways

Cellular respiration may be achieved with or without oxygen, aerobically or anaerobically. Aerobic respiration is the most efficient form of energy recovery; for each molecule of glucose broken down, 38 ATP molecules are generated. The overall reaction looks like this:

$$\text{glucose} + \text{oxygen} \longrightarrow \text{carbon dioxide} + \text{water} + \text{energy; or,}$$

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{energy}$$

The oldest and most common respiratory pathway is that of anerobic respiration or fermentation. In fermentation each molecule of glucose yields only 2 molecules of ATP. Many aerobes retain the ability to ferment, but most of them have lost the ability to dump the end products of fermentation. The end products of fermentation are energy, carbon dioxide, and either alcohol or lactic acid. For example, the muscle cells of humans can ferment (make energy by respiring without oxygen) in emergencies, but the build up of lactic acid and ethyl alcohol produces fatigue and muscle cramps. Human cells must aerobically respire in order to change wastes to carbon dioxide and water.

Some oganisms can function as either aerobes or anaerobes. They are called facultative anaerobes. Yeasts and many bacteria are anaerobes. They can maintain themselves without oxygen. When oxygen becomes available, they can aerobically respire and burn their accumulated biochemical garbage (lactic acid or alcohol), using the burning to produce extra energy. At these times they can multiply very fast.

The purpose of the following procedure is to measure the relationship between available food and the amount of energy released by a simple organism, a yeast.

Pre-lab

Collect the following equipment and materials.

Equipment

distilled water
glass stirring rod
marking pen
millimeter ruler
test tube rack
test tubes, 13 × 100 mm.
(10 per group)
test tubes, 22 × 175 mm.
(10 per group)
100 ml. graduated cylinder
125 ml. erlenmeyer flask
250 ml. erlenmeyer flask
10 ml. pipets

Materials

dry yeast
molasses

Special Preparations

1) The [illegible] × 100 mm. test tubes should be lipless. They will serve as large durham tubes and fit upside down inside the large test tubes.

2) Make a stock yeast suspension for the class by dissolving a package, or 1 tablespoon, dry yeast in 1 liter warm water.

3) It is best to use warm (**not hot**) molasses in the laboratory. Students have less chance of becoming sticky during their measurements.

Time Required

This respiration laboratory requires two classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need all materials and equipment listed in the Pre-lab.

Studies using baker's or brewer's yeast have provided much of our present knowledge of carbohydrate metabolism. Both are varieties of *Saccharomyces cerevisiae*. This yeast derives its name from an early observation that it is a sugar consuming fungus, therefore, the name *Saccharomyces* (*saccharum* = sugar, and *myces* = fungus).

Common yeast is a facultative anaerobe. When maintained under anaerobic conditions, *S. cerevisiae* forms alcohol and carbon dioxide from sugar. This process is called an ethanolic or alcoholic fermentation. The overall equation is:

$$\text{Sucrose} + \text{water} \xrightarrow{\text{yeast}} \text{ethanol} + \text{carbon dioxide} + \text{energy; or,}$$

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{yeast}} 4CH_3CH_2OH + 4CO_2 + \text{energy.}$$

It is easy to measure the amount of carbon dioxide production. Since carbon dioxide production is proportional to energy production, measurement of carbon dioxide production indirectly yields a figure for energy production.

Steps

A. Number the large test tubes from #1 to #10.

B. Prepare a yeast suspension by adding 30 ml. of the stock yeast suspension to 70 ml. distilled water in a 125 ml. flask.

C. Prepare, by serial dilution in the ten large test tubes, a series of molasses concentrations from very high in test tube #10, to very low in test tube #1. This is done as follows:

1). Measure 25 ml. molasses and pour it into test tube #10.

2). Measure another 25 ml. molasses into the graduated cylinder and add 25 ml. distilled water. Shake the mixture, stir it with a glass rod, and pour it back and forth from the graduated cylinder to a flask until it is evenly mixed. Pour 25 ml. of the mixture into test tube #9. Save the remaining 25 ml. in the flask.

3). Measure 25 ml. distilled water into the graduated cylinder and add the molasses/water you left in the flask. Again, mix it together completely, and pour 25 ml. of the mixture into test tube #8. Save the remaining 25 ml. in the flask.

4). Repeat this operation until all ten test tubes are filled with 25 ml. each of varying dilutions of molasses/water. Discard the remaining 25 ml. mixture from the flask.

The molasses concentration in the ten test tubes will be:

#10 = 100%
#9 = 50%
#8 = 25%
#7 = 12.5%
#6 = 6.25%
#5 = 3.13%
#4 = 1.57%
#3 = 0.78%
#2 = 0.39%
#1 = 0.20%

D. Thoroughly shake the flask containing the 100 ml. yeast suspension, and pipet 5 ml. yeast suspension into each test tube, #1 through #10. Thoroughly mix the yeast suspension with the molasses and water mixture in each test tube, by placing your thumb over the test tube mouth and shaking vigorously. Clean and dry your thumb before proceeding to the next test tube to avoid any mixing of concentration ratios.

E. Put a small test tube upside down into each of the ten large tubes. It may be easier (particularly with test tube #10) to carefully fill the small test tube to the brim with the contents of the large test tube, and then to quickly upend it into the larger test tube. Repeat the procedure until there are no air bubbles left inside the small tube. If this is impossible (depending upon the thickness of the molasses), carefully measure the length of the air bubble with a millimeter ruler and record the measurement.

F. Carefully place your labeled test tube rack with its test tubes #1 through #10 in a convenient area and do not disturb for 24 hours.

Procedure: Hour 2

Students will need all materials and equipment set up during hour 1.

Steps

A. Observe what has taken place in the 24 hours since the experiment started.

1. What do you see? ____

____

____

B. Measure the quantity of gas collected in the top (actually the bottom) of each small test tube by measuring the height of the gas column with a millimeter ruler.

2. Record the height of the gas column in test tubes #1 through #10 in figure 1 below.

#	gas column height
1	
2	
3	
4	
5	

#	gas column height
6	
7	
8	
9	
10	

Figure 1

3. Since the diameter of the small test tubes is known (13 mm.), it is easy to determine the volume of gas in the cylinder after measuring the gas column height. Find the area of the test tube ($A = \pi r^2$) and then the volume ($v = \pi r^2 \times$ height). Record the quantity of gas collected in each test tube in figure two below.

#	gas quantity
1	
2	
3	
4	
5	

#	gas quantity
6	
7	
8	
9	
10	

Figure 2

4. Plot the different concentrations of molasses (test tube #1 through #10) versus the production of carbon dioxide in 24 hours on the following graph, figure three.

Figure 3

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5. What relationship seems to exist between the concentration of available food and the production of carbon dioxide by yeast cells?

6. What sources of error do you note in the set up of this laboratory?

7. How can you correct for these discrepancies?

8. Molasses is a "complex" material containing 60% sucrose with smaller amounts of glucose and fructose, amino acids, sulfur dioxide, and vitamins. How could you design a laboratory that would test which one of the several materials which constitute molasses is responsible for the most energy and carbon dioxide production?

Optional Activities

Students may wish to explore some variables concerning energy production by yeast. They can submit their results to the instructor in a formal laboratory report.

1). Students can repeat the above experiment but use sucrose, or glucose, or fructose, instead of molasses. They can then compare the results with those obtained from molasses. They can alternately run the experiment using a mixture of the three sugars.

2) Students can repeat the above experiment under different temperature conditions. They can then consider the impact of temperature on yeast activity.

3). Students can obtain brewer's yeast, or wild yeast, and repeat the above experiment using a different variety of yeast.

Resources

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Phaff, Herman J. "Industrial Microorganisms." *Scientific American*. September 1981.

Rose, Anthony H. "The Microbiological Production of Food and Drink." *Scientific American*. September 1981.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

aerobe
anaerobe
facultative anaerobe
fermentation
glycolysis
respiration

Review

9. Respiration & Fermentation

Name____________________________

Date____________________________

Short answer

1 Design an experiment which measures the respiration rate of a living plant or animal.

2 Define fermentation and give its starting materials and end products.

3 Describe how the processes of photosynthesis, glycolysis, and respiration all interact in living systems.

Answers Found: Essay 1: (thought); Essay 2: p 87 - 88; Essay 3: (thought).

10. Discussion

Electrophoresis

When you have completed this discussion you should be able to:

1. Describe electrophoretic processes.
2. Discuss the different physical and chemical factors affecting electrophoretic data and results.
3. Give examples of research applications of electrophoresis.

Definition

Electrophoresis is a separation technique which utilizes the fact that substances move at different rates when subjected to an electrical current, depending upon their molecular size and electrical charge. Electrophoresis is a useful and relatively simple technique for the separation and study of the properties of single charged molecules, particularly proteins and nucleic acids.

Background

Sickle Cell Anemia and Electrophoresis

Linus Pauling, an American chemist, used the techniques of electrophoresis to study the structure of hemoglobin. Pauling reasoned that a disease such as sickle cell anemia might reflect a deviation in the protein structure of the hemoglobin molecule. Pauling built upon a Nobel Prize winning discovery in 1941 by two colleagues, George Beadle and Edward L. Tatum, of the California Institute of Technology. They had demonstrated that a mutation of one gene could be correlated with the loss of function of one enzyme. Pauling applied this idea of one gene-one enzyme (or polypeptide chain) to his theory of sickle cell anemia as a hemoglobin mutation. He took samples of hemoglobin from people with sickle cell anemia, from others heterozygous for the gene, and from still others homozygous for the normal gene. Pauling dissolved the three types of hemoglobin in a solution and subjected them to a weak electric field. Their electrophoretic separation curves were different, with normal hemoglobin being the most negatively charged. This difference had not previously been demonstrated.

A few years later Vernon Ingram, of Cambridge University in England, was able to show that the actual difference between normal and sickle cell hemoglobin molecules is only one amino acid in three hundred.

Linus Pauling's results shed new light not only on sickle cell anemia but also on the entire concept of genetic dominance and recessiveness. Note in the following chart, which illustrates Pauling's electrophoretic results, that the individual heterozygous for the trait actually produces both types of hemoglobin but that there is enough of the normal type to prevent the individual from displaying the disease. The three types of hemoglobin formed definite, distinct reactions to the electric field, pointing generally to what took years of biochemical research to prove.

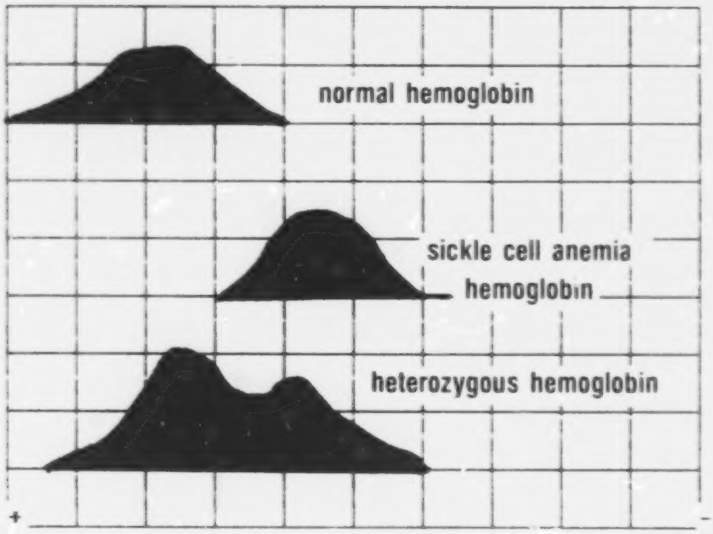

Figure 1

Protein Mobility and Archeology

All proteins have an electric charge determined by their amino acid composition, and when in solution, they can move in an electric field. An estimate of structural differences between proteins can be made from the amount of difference in their mobility. Some proteins, such as collagens of bone and skin, keratins, and seed proteins, are often preserved over time, allowing electrophoretic techniques to be used on fossil materials. Such results are used to trace phylogeny.

Genetic Variability

Analysis at the molecular level is one method for assessing genetic variability. Such variability is characteristic of nearly all natural populations, and it is useful to be able to quantify similarities and differences. Electrophoretic techniques can be used to measure allelic differences in organisms which are difficult to breed, because electrophoresis does not require direct analysis of the genes themselves. Literally hundreds of species have been analyzed electrophoretically. The results demonstrate the extensiveness and universality of genetic variation.

Medicine

The medical field utilizes electrophoretic techniques in several ways. For example, it has been used to study the defect in which some people produce excessive numbers of plasma lipoproteins. A lipoprotein is a multicomponent complex of protein and lipids of a characteristic density, molecular weight, size, and chemical composition. These complexes of protein and lipid are held together by noncovalent forces. While a typical chemical composition and molecular weight exists for each type of lipoprotein complex, there may exist no exact structural integrity. Lipoproteins serve a wide variety of functions in cellular membranes and in the transport and metabolism of lipids. Plasma lipoproteins transport lipids from the sites of their absorption into blood to the various tissues of the person where lipids are utilized.

Through electrophoresis four classes of plasma lipoproteins have been identified in fasting humans. Through the separation and classification of these lipoproteins with electrophoretic technology, doctors are now able to note the side effects which can occur in humans having too many circulating lipoproteins. They can suffer from increased risk of atherosclerosis and high levels of cholesterol, as well as diabetes mellitus and chronic pancreatitis.

Electrophoretic Techniques

The underlying principle of electrophoresis is that any charged particle suspended between the poles of an electric field tends to travel toward the pole bearing the charge opposite its own. The rate at which it travels is conditioned by such factors as the characteristics of the particle itself, the properties of the electrical field, and the nature of the medium it is traveling through. Its mobility is also affected by such environmental factors as temperature and humidity. Generally, however, the mobility of a particle is proportional to its charge/mass ratio.

Electrophoretic mobility is the rate at which a particle moves under a controlled set of circumstances. Because of the variabilities noted above, results are comparable only when derived from the same, or same type of electrophoretic instrument and similar environmental conditions. In other words, absolute quantities can not be derived, but comparative results can be realized.

The area upon which electrophoretic separation occurs is called the bed. The bed can be composed of any number of materials, including gels, films, and powders. It is moistened with an electrolytic solution, usually buffered, and the ends of the bed are immersed in electrolyte contained in two chambers. The chambers are designed to hold electrodes connected to a direct current power source. The electrolyte level in the electrode chambers must be held stable so that siphoning from the bed does not invalidate results. Also, the entire apparatus must be held in an airtight chamber to minimize evaporation of buffer.

There are two basic types of electrophoresis, free solution electrophoresis and zone electrophoresis.

Free Solution Electrophoresis

In free solution electrophoresis, the sample to be analyzed is dissolved in a liquid, and the sample is fractionated in a U tube. First the tube is filled with unstabilized buffer. The sample then is injected into the system through a capillary tube sidearm at the bottom of the U tube. An elaborate optical system locates and measures movement of sample fractions through the tube.

There are many cost and technical difficulties in maintaining free solution electrophoresis set ups, such as maintaining careful thermal regulation and avoiding vibration interference. Because of this, electrophoretic techniques which employ stabilization media such as gels are more often used. Nevertheless, free solution electrophoresis is more precise and remains the standard reference for measuring electrophoretic mobilities.

Zone Electrophoresis

In zone electrophoresis, stabilizing media are used which separate sample material into discrete zones, or bands. These zones can be snipped out and analyzed. A wide variety of materials have been used. Filter paper, cellulose acetate strips, starch powder, cellulose powder, ion exchange paper, glass microbeads, starch gel, agar gel (also known as agarose), and synthetic gels such as polyacrilamide gel have all been used as media in zone electrophoresis.

An important technical variation on zone electrophoresis is that of immunoelectrophoresis. Immunoelectrophoresis is a technique whereby biological materials are separated into fractions by zone electrophoresis, and the fractions are allowed to react with immune serum after diffusing into the electrophoretic bed. Since this adds a very specific detection technique (a precipitin reaction) to electrophoresis, it is possible to separate many additional fractions in a protein mixture.

When the purpose of an electrophoretic procedure is more to prepare a significant amount of purified substance for analysis, rather than simple detection, the capacity of the bed can be increased by layering it thickly with some absorbent material, such as starch powder or cotton.

Resources

Darnell, James. "The Processing of RNA," *Scientific American*, June 1984.

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Lawn, Richard M. and Gordon A. Vehar. "The Molecular Genetics of Hemophilia," *Scientific American*, March 1986.

Tanaka, Toyoichi. "Gels," *Scientific American*, January 1981.

Weber, Klaus, and Mary Osborn. "The Molecules of the Cell Matrix," *Scientific American*, October 1985.

Terminology

Students should understand the following terms and concepts prior to taking the unit review.

bed
electrophoretic mobility
electrophoresis
free solution electrophoresis
genetic variation
immunoelectrophoresis
lipoprotein
sickle cell anemia
zone electrophoresis

Review

10. Electrophoresis

Name

Date

Define the following

1. Free solution electrophoresis
2. Zone electrophoresis
3. Immunoelectrophoresis
4. Lipoprotein
5. Electrophoretic mobility

Essay

1. Explain how electrophoresis aids in the study of genetic variability.

2. Describe the different physical factors which prevent electrophoretic results from having quantifiable, absolute values for reference.

3. Discuss the significance of the electrophoretic results Linus Pauling obtained by comparing normal, heterozygous, and sickle cell hemoglobin.

Answers Found: p 98 - #5; p 99 - #1, 2, 3, & 4; Essay 1: p 98; Essay 2: p 99; Essay 3: p 97.

11. Laboratory

Gel Electrophoresis

When you have completed this laboratory you should be able to:

1. Explain the process of gel electrophoresis.
2. Achieve the separation of lactic dehydrogenase isoenzymes on agarose slabs.
3. Incubate LDH isoenzyme gel to demonstrate the reactions of the enzyme in specially prepared media.
4. Visually show zones of LDH activity on agarose gel.
5. Explain the applications and techniques of isoelectric focusing in gel electrophoresis.

Purpose

Electrophoresis utilizes the fact that charged molecules in solution migrate in response to an electrical field. If executed with care and precision, electrophoresis can yield valuable information about those molecules. In this laboratory the student will have an opportunity to practice electrophoretic technique by separating isoenzymes.

Amphoteric Proteins

Most proteins are amphoteric compounds. That means that they contain both acidic and basic residues and that their net charge is determined by the pH of their medium. Each protein has its own characteristic properties, depending upon the number and kind of amino acids of which it is composed. Amino acids possess both carboxyl ($\text{-COOH} \longleftrightarrow \text{-COO}^- + \text{H}^+$) and amino groups ($\text{-NH}_2 + \text{H}^+ \longleftrightarrow \text{-NH}_3^+$), and these groups become electrically charged when ionized.

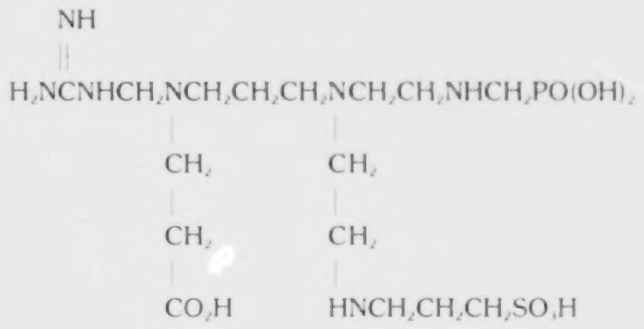
Isoelectric Points

Amphoteric proteins have a pH at which there is no net electrical charge. This pH is called the isoelectric point. Just as proteins have their own characteristic properties, so too do they have their own isoelectric points. For example, normal human hemoglobin has an isoelectric point at pH 7.07. Above the isoelectric point of a protein, the protein will have a net negative charge, and it will migrate toward the anode in an electrical field until it reaches its isoelectric point. Below the isoelectric point, the protein will have a net positive charge, and it will migrate toward the cathode. The pH of solutions in an electrophoretic system must be kept constant to maintain a steady charge and protein mobility. To do this the solutions must be buffered.

Nucleic acids are exceptions. Although they are proteins, they are not amphoteric and remain negative at any pH.

Isoelectric Focusing and Ampholytes

Isoelectric focusing is a special electrophoretic technique that uses special buffers in the separation of proteins which differ but slightly in their isoelectric points. These buffers are called ampholytes. They maintain a constant pH when encountering a fairly wide range of pH. This pH stability allows slightly different proteins to move to separate areas within the gel. Ampholytes may consist of several buffers combined together. Many carrier ampholytes are based on aliphatic polyamines reacted with sulfo, phospho, and carboxyl groups. Below is an example of a typical carrier ampholyte.



$$\begin{array}{llll}
NH & & & \\
\| & & & \\
H_2NCNHCH_2N & CH_2CH_2CH_2N & CH_2CH_2NHCH_2PO(OH)_2 & \\
 & | & | & \\
 & CH_2 & CH_2 & \\
 & | & | & \\
 & CH_2 & CH_2 & \\
 & | & | & \\
 & CO_2H & HNCH_2CH_2CH_2SO_3H &
\end{array}$$

Figure 1

Materials

The electrophoresis performed in this laboratory will be run on a horizontal agarose gel slab. Agarose, a gel derived from seaweed, is the easiest gel to formulate and still obtain effective results. Also, polyacrylamide gels, although often used industrially, are highly neurotoxic and not safe for beginning students. Agarose gels also have larger pore sizes than polyacrylamide gels, and therefore the electrophoretic process is accomplished much more rapidly. The proteins to be analyzed are lactic dehydrogenase isoenzymes (LDH).

Lactic Dehydrogenase Isoenzymes

Isoenzymes are similar enzymes which can catalyze the same reaction but which have different physical properties and react differently in an electrical field. Human isoenzymes which have been studied in conjunction with disease are lactic dehydrogenase, creatine kinase, and alkaline phosphatase. These isoenzymes are found in tissues throughout the human body but are more concentrated in some tissues than in others.

Lactic dehydrogenase is a tetrameric enzyme (a molecule consisting of four structural subunits or peptide chains), but only two distinct subunit origins have been found. These two subunits are configured five different ways in humans. The five types of human LDH are found primarily in the myocardium of the heart, red blood cells, the brain, the kidney and liver, and in skeletal muscle.

Medical researchers have developed keen interest in the levels of lactic dehydrogenase and creatine kinase in people who have suffered heart attacks. The levels of both isoenzymes increase sharply after an attack, and elevated lactic dehydrogenase (LDH) activity can be demonstrated for more than two weeks later. LDH isoenzyme levels can be traced in the blood by electrophoretic isoelectric focusing on agarose gels. Creatine kinase is released immediately after cellular damage to the heart, but LDH release lags by 1 - 2 days. Normally doctors can identify what type of heart attack occurred by identifying the amount and the type of LDH which is predominant.

Infarctions, secondary liver congestion, and other complications of a heart attack can be monitored through electrophoresis of the five LDH types. LDH increases also can be observed in progressive muscular dystrophy, liver diseases such as infectious hepatitis, leukemia, certain anemias, and in renal disease. Although it is known that these isoenzyme increases occur, it is still not known exactly why they occur or what these chemical changes reveal about bodily mechanisms.

Monitoring LDH Activity Levels

After separation it is possible to incubate LDH electrophoresed gels in a medium which promotes a dyeing reaction in the area of LDH activity, thereby allowing simple visual inspection of the results. The following set of reactions can be obtained by incubation of LDH electrophoresed gels (these reactions will be used in hour three of this laboratory):

$$\text{Lactate} + \text{NAD} \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH}$$

$$\text{NADH} + \underset{\text{(oxidized)}}{\text{Phenazine Methosulfate}} \longrightarrow \text{NAD} + \underset{\text{(reduced)}}{\text{Phenazine Methosulfate}}$$

$$\underset{\substack{\text{Methosulfate}\\ \text{(reduced)}}}{\text{Phenazine}} + \underset{\text{Tetrazolium}}{\text{Tetranitroblue}} \longrightarrow \underset{\substack{\text{Tetrazolium}\\ \text{Formazan}}}{\text{Tetranitroblue}} + \underset{\substack{\text{Methosulfate}\\ \text{(oxidized)}}}{\text{Phenazine}}$$

Tetranitroblue tetrazolium formazan is a purple precipatate which is very brightly colored and insoluble. It deposits on the gel in the areas of high LDH activity, thereby allowing the student to visually scan the slab for evidence of positive experimental results.

Process

During this laboratory the student will determine the characteristic electrophoretic patterns of lactic dehydrogenase isoenzymes and also separate and identify the components of these isoenzymes using the electrophoretic technique of horizontal isoelectric focusing.

Pre-lab

Supplies needed for gel electrophoresis.

Equipment

horizontal electrophoresis unit with 0.5 or 0.75 mm. spacers
power supply with constant voltage and wattage
casting stand
2 glass plates per unit
2 clamps per unit
pH meter
boiling water bath or microwave
10 ml. pipets
glass tube or roller
large test tube
hair dryer or other hot air source
plastic tray
test tubes
50° C. water bath
250 ml. graduated cylinder
50° C. oven
refrigerator
scissors
37° incubator

Materials

The enzyme, buffers, gel, and stains for this laboratory must all be refrigerated immediately at 0° to 5° C.

lactic dehydrogenase isoenzyme
agarose gel
distilled water
absorbent paper
blotter paper
solution A
solution B
NAD (nicotinamide adenine dinucleotide)
electrophoresis buffer (ampholyte)
electrophoresis plastic film
10% glycerol
filter paper
sorbitol
salt solution (dilute)

Some of these materials must be prepared in advance, and some can be ordered only from a limited number of suppliers.

Special Preparations

1) Electrophoresis units: agarose gel may be used on both horizontal and vertical electrophoresis units, but this laboratory is designed for a less expensive horizontal unit. Each student group will need one unit. Power supplies which can run multiple slab units are available.

2) LDH isoenzyme kits: LDH isoenzyme electrophoretic kits are commercially available. For example, Sigma kit #705-G has sufficient materials for five student groups, including agarose gel, buffers, and stains. On the other hand, the teacher may prefer preparing many of these materials, as follows:

3) **Do not forget, all kits, enzymes, buffers, and stains must be stored in a refrigerator set at 0° to 5° C.**

4) Purchase lactic dehydrogenase isoenzymes, LD 1 - 5 (the five forms of LDH), freeze dried in human serum matrix.

5) Solution A: mix 1 ml. 70% sodium lactate, 1.87 g. $Na_2HPO_4 \cdot 2H_2O$, 0.272 g. KH_2PO_4, 25 mg. tetranitroblue tetrazolium, 50 mg. sodium cyanide, and sufficient distilled water to total 90 ml. Buffer the solution to pH 7.4 and refrigerate it.

6) Solution B: mix 1 mg. PMS (phenazine methosulfate) per 1 ml. distilled water. Refrigerate the solution in a dark bottle. 1 ml. of solution B will be used for every 36 ml. of solution A.

7) 10% glycerol: measure 10 ml. glycerol and add distilled water to make 100 ml. of solution.

Time Required:

The gel electrophoresis laboratory requires three classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the following: casting stand, glass plates, clamps, water baths, oven, refrigerator, graduated cylinder, pipet, large test tube, absorbent paper, electrophoresis film, 10% glycerol, and LDH kits (or agarose, sorbitol, ampholyte, and distilled water).

During this first hour students will set up the apparatus upon which the experiment is to be run and gain familiarity with electrophoresis equipment and techniques.

Steps

A. Melt the agarose solution in the LDH kit in a boiling water bath or microwave, following directions in the kit. If kits are not available, make a solution of 1.0 gram agarose, 12.0 grams sorbitol, and enough distilled water to make 100 ml. solution. Hold the solution in a 50° C. water bath.

B. Fill a 250 ml. graduated cylinder with distilled water and a 10 ml. pipet, and place it in the same water bath. Also place a large test tube in the 50° C. water bath. Keep the glassware in the bath until used.

C. Lay one of the glass plates on a flat surface. Squirt a line of 10% glycerol up the center of the plate.

D. Cut a 12 × 13 cm. piece of electrophoresis film. Hold the film by its 12 cm. edges with the gel binding side facing you (the gel binding side will be on the inside of the roll).

E. Place the center of the film, the side opposite the gel binding side, on the plate to which you have applied a line of glycerol, and fold down the edges, taking care that no air bubbles are trapped. The bottom edge of the film should be 1 - 2 mm. from the bottom edge of the plate, and the sides equidistant from the sides of the plate.

F. Place absorbent paper over the plate and roll with a roller or glass tube. This will bond the film to the glass and remove excess glycerol solution from behind the film.

G. Place the spacers along the 16 cm. edges of the glass plates. If the spacers cover any of the film, reposition the film so that it is located between the spacers.

H. Place the second plate over the spacers and clamp the whole thing together, taking care that the clamps are properly aligned. This is called the "sandwich." Cam the sandwich to the casting stand and place it all in a 50° C. oven. Leave in oven until step J.

I. Pipet the correct amount of ampholyte (in kits sometimes labeled as agarose buffer) and agarose solution into the test tube. The correct amounts depend on the size spacers used. If the gel thickness is to be 0.5 mm., add 0.63 ml. ampholyte and 10 ml. agarose solution. If the gel thickness is to be 0.75 mm., add 0.94 ml. ampholyte and 15 ml. agarose solution. In both cases the ampholyte will be 40% of the solution volume. Mix.

J. Remove the casting stand and sandwich from the oven and quickly pipet the agarose/ampholyte mixture into the sandwich.

K. Allow the sandwich to come to room temperature and then place the assembly in the refrigerator overnight, or until performing the second hour of the procedure.

Procedure: Hour 2

Students need the following: prepared casting stand assembly from hour one procedure, blotter paper, paper toweling or filter paper, electrophoresis unit, power supply, LDH isoenzymes, pipet, plastic tray, LDH kit (or NAD and solutions A and B), and scissors.

In this hour you will electrophorese the gel.

Steps

A. Open the glass sandwich. Lift the film with gel off the glass plate. The gel should remain stuck to the plastic film; if it does not, repeat hour one. Trim approximately 3 mm. from all four edges of the film so that the gel thickness on the remaining film is uniform.

B. Position the film, with gel side up, on the running surface of the electrophoresis unit. Adjust the temperature of the unit to 10° to 15° C.

C. Load the LDH isoenzyme samples onto the middle of the gel. If using a kit, follow the directions. Otherwise, use freeze dried LDH isoenzyme, 0.5 ml., which has been dissolved in distilled water, 2.5 ml. Pipet one drop of the solution onto thin paper or filter paper; carefully cut out the wet dot and place it on to the gel. Repeat five times so that a row of five dots runs down the middle of the gel parallel to where the electrodes will be placed.

D. Place the surface electrodes directly on the surface of the gel. Assure that the wires run on the gel about 3 mm. from the edges. Directions for this portion of the procedure might vary depending upon the brand of gel electrophoresis unit. Read the manufacturer's directions.

E. Place small triangles of blotter paper under the electrodes at the points where the wires come off the edges of the gel. Position the triangles so that a tip extends away from the gel. These "wicks" will absorb liquid as it accumulates at each electrode and carry it away from the gel.

F. Run the gel at 15° C. at 5 watts and 200 volts for 5 minutes. Then increase the voltage to 1000 for 10 minutes. If pools of liquid appear on the surface of the gel while it is running, turn the power supply off and lightly blot the liquid from the surface of the gel. After a total running time of 15 minutes, turn the power supply off and remove the paper dots. Continue the run for another 10 minutes at 5 watts and 1000 volts.

1. It is during the run at 200 volts that the ampholytes arrange themselves according to the pH they buffer. During operation at 1000 volts what happens to the LDH proteins?

G. While the gel is running, pour solutions A and B and NAD into a plastic tray, per the following proportions. For every 36 ml. of solution A, use 1 ml. of solution B. Add 4 mg. NAD (nicotinamide adenine dinucleotide) for every 37 ml. of solutions A and B.

H. When the gel has been electrophoresed, carefully turn the power off and remove the gel. Do not remove the plastic film backing or disturb the gel. Carefully place it in the plastic tray with solutions.

I. Incubate the gel at 37° C. for at least 2 hours, or until hour 3 of this laboratory.

Procedure: Hour 3

Students need the following: the incubated gel run during hour two, hair dryer or other hot air source, glass plate, pH meter, test tubes, dilute salt solution, scissors, filter paper, and absorbent paper.

Steps

A. Remove the incubated gel from the plastic tray. **Do not remove the film backing on the gel.** Dry the gel by first placing it on a flat surface and covering it with a sheet of filter paper. Place about a half inch pack of absorbent paper on the filter paper, followed by a glass plate, and then a heavy textbook. Leave the pile approximately 20 minutes.

B. Remove the gel and finish drying it with a hair dryer.

2. What do you see? ______________________________

3. In the space below, make a diagram of your gel, labeling dyed points, their intensity, size, banding, and position. Label the distance, in millimeters, of each dyed area in relationship to its originating dot.

4. How many isoelectric points originate from one LDH dot? __________

5. Are the isoelectric points similar in their distancing from the LDH dot origin? Why or why not?

____

____

____

6. It is possible to read the pH gradient of LDH manually. Slice the gel and sequentially place a portion of each dyed band in separate test tubes containing a small amount of dilute salt solution. Incubate the test tubes overnight as the ampholytes slowly diffuse out into the salt solution. It will take but a moment the next day to determine the pH of each solution with a pH meter. Record the pH and the distance from the originating dot of each dyed area.

dyed area	distance from origin	pH
origin	0	
1		
2		
3		
4		
5		

Figure 2

Resources

Gordon, A. H. "Electrophoresis of Proteins in Polyacrylamide and Starch Gels," *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 1, North-Holland Publishing Co.: Amsterdam-London, 1972.

Terminology

Students should understand the following terms and concepts prior to taking the unit review.

- agarose
- ampholyte
- amphoteric protein
- gel
- gel electrophoresis
- isoelectric focusing
- isoelectric point
- lactic dehydrogenase (LDH) isoenzyme
- tetrameric

Review
11. Gel Electrophoresis

Name____________________

Date____________________

True or false: If the statement is true, mark it true. If the statement is false, replace the incorrect italicized term with the correct term.

______ 1. Tetranitroblue tetrazolium is a *buffer*. ____________________

______ 2. The anode has a *negative* charge. ____________________

______ 3. LDH is a typical *buffer*. ____________________

______ 4. The net charge of amphoteric compounds is determined by the pH of their *medium*. ____________________

______ 5. Buffers which can carry a wide range of pH are called *isoelectric*. ____________________

Short answer

1. Why is a colored insoluble precipitate formed from tetranitroblue tetrazolium in the presence of LDH activity?

2. What structure(s) causes proteins to exhibit amphoteric reactions? Why?

Short answer

3. What are the advantages of isoelectric focusing in gel electrophoresis?

Answers Found: p 103 - #2, 4, & 5; p 104 #3; p 105 - #1; Short Answer 1: p 105; Short Answer 2: p 103; Short Answer 3: p 103 & 104.

12. Laboratory

Cells

When you have completed this laboratory you should be able to:

1. Demonstrate the proper use of a compound microscope.
2. Explain how a prokaryotic cell differs from a eukaryotic cell.
3. List structures found in unicellular organisms that are not usually found in multicellular organismal cells.
4. Describe the structures of both a typical plant and a typical animal cell as seen with a light microscope.
5. Describe specialized adaptations found in multicellular organismal cells.

The Microscope

Scientific advances often have been achieved by the development of instruments capable of extending the range of observation. The light microscope is one such instrument, and its development engineered a major change in the biological sciences by opening up the world of cells. In this laboratory you will be introduced to this world.

The human eye is unable to detect objects with a diameter less than approximately 0.004 inch, or 0.1 millimeter. The very existence of cells was unknown until invention of the microscope at the beginning of the seventeenth century. Early microscopes were of two kinds. The first contained a single lens of very short focal length. Such instruments are similar to magnifying glasses, which have been known since antiquity. The second was the compound microscope, with a double lens system consisting of an ocular and objective and capable of far greater power of magnification. All contemporary light microscopes are of the compound type.

There are many variations possible on a basic compound microscope. A typical one might appear as below:

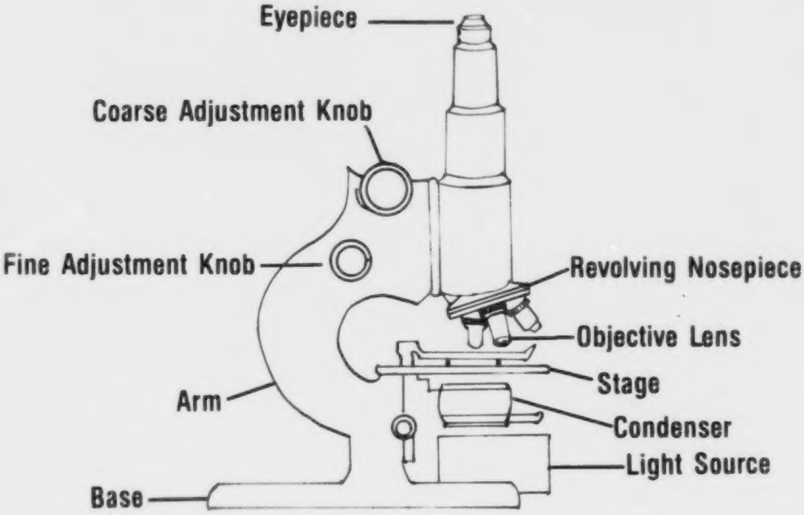


Figure 1

Many variations on the compound microscope are possible. The eyepiece can be monocular or binocular, there can be several objectives of varying powers of focus, the light source can be a simple mirror or electric bulb, etc. One can design a light microscope for use with polarized light, dark field fluorescence, phase contrast, and a number of other types of microscopy that show some cell components very clearly. Oil immersion microscopes have a special objective which can be used by placing a drop of oil on the slide; it increases the magnification of the microscope as the objective pulls the oil upwards by surface tension.

Still other variations include changes to the stage of the microscope so that it can hold dishes of organisms. At low power enlargement these stage-varied instruments are simple dissecting microscopes. When designed for high power enlargement of individual cell cultures, they become complex and expensive inverted microscopes. Additionally, any light microscope can be designed to hold cameras and other attachments.

The Electron Microscope

It was not until invention of the electron microscope after WWII that there was a significant breakthrough in microscope technology. Rather than using light, as in the compound microscope, the electron microscope uses a beam of electrons emitted by a tungsten filament to form an image of the specimen. The glass lenses of the light microscope are replaced by electric fields, and the image is viewed on a fluorescent screen. Since the electrons will form a coherent beam only when traveling in a vacuum, it is not possible to introduce living cells into the column of an electron microscope. The resolving power of the electron microscope is much greater than that of the light microscope. The electron microscope is capable of magnifications as high as 160,000 times. The micrographs taken with the electron microscope may be further enlarged photographically to achieve magnifications in excess of 1,000,000.

Pre-lab

Supplies needed for this laboratory:

Equipment

microscope
cover slips
razor blades
forceps
microscope slides
teasing needles
droppers

Materials

leaves, any type
Paramecium, living culture
methylene blue
10% methyl cellulose
crystal violet
toothpicks
sterile lancets
70% alcohol
yogurt or cottage cheese
congo red stained yeast cells
acetocarmine
lens paper
immersion oil
Euglena, living culture
cotton balls
prepared slides of the human intestine, a leaf in cross section, and the three bacterial types

Special Preparations

1) *Paramecium*: aerate the culture upon arrival and store in subdued lighting at 60 - 70° F. *Paramecium* cultures are usually very concentrated and easy to subculture. For short time storage add one wheat grain to each jar of *Paramecium*.

2) Euglena: also aerate upon receipt and keep at 60 - 70° F. However, place in sunshine or other bright light.

3) Put the stains and other chemicals in dropper bottles for ease of use.

4) 45% acetocarmine: add 45 ml. glacial acetic acid to 55 ml. distilled water in a graduated cylinder. Add 0.5 g. carmine and boil 3 minutes. Cool, filter, and place in stock bottle. Dilute one part of the stock with two parts 45% acetic acid before use.

5) Methylene blue: dissolve 1.6 g. methylene blue in 100 ml. 95% ethanol. Dilute (1:10) 10 ml. of the mixture with 90 ml. distilled water before use.

6) Methyl cellulose: use protoslo, or 10% methyl cellulose. In the latter case, mix 10 g. methyl cellulose with 90 ml. distilled water and allow the solution to mix on a magnetic stirrer until the cellulose is completely dissolved and the viscosity uniform.

7) Congo red stained yeast cells: add 1 g. yeast cells to 20 ml. distilled water. Boil 5 minutes. Add a toothpick full of congo red and stir. Allow 15 minutes for the dye to penetrate into the yeast cells. Refrigerate until ready for use. Do not keep more than two days because the suspension spoils rapidly.

8) Leaves: literally any thin leaf type will do. *Elodea* or *Anacharis* leaves are fine if a fish tank is handy; lettuce or Italian (flat) parsley works perfectly well also.

Time Required:

The cells laboratory requires three classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the listed equipment, as well as the following materials: cottage cheese or yogurt, toothpicks, crystal violet, immersion oil, and prepared slides of the three bacterial types.

Prokaryotic Cells

During hour one students will view the simplest cells of all, prokaryotic cells. Prokaryotic cells belong to the kingdom *Monera*, which comprises both the bacteria and the blue-green algae. Prokaryotes lack the organelles of true cells, the eukaryotes. The DNA of a prokaryote exists as a single, naked molecule floating in the cytoplasm of the cell. Prokaryotes contain ribosomes, but the ribosomes float in the cytoplasm, or clump together in groups known as polysomes. All members of the kingdom *Monera* have cell walls, and many have special structures for movement, such as long, whip-like flagella. Prokaryotic cytoplasm may include capsules, gas vacuoles, and other inclusion bodies. Many contain chlorophyll for photosynthesis.

In the kingdom *Monera* the bacteria belong to the division *Schizomycetaceae* (which means "fission fungi"). Bacteria are spread universally and consist of almost numberless species.

In this laboratory hour you will study three different types of bacteria. Because it is difficult to ascertain structural differences among bacteria, bacteria are classically grouped according to morphology — shape, stainability, and flagellation. Further, since the early nineteenth century, three basic shapes have been used to describe bacteria — round, rod, and spiral.

Fermentation of Dairy Products

Fermentation has been used since the dawn of civilization as a mechanism to prevent dairy products from spoiling. The general procedure is to introduce microorganisms which convert the milk sugar, lactose, into lactic acid. This results in a pH drop and the formation of curd due to the coagulation of casein. The lower pH prevents the growth of unfavorable bacteria which would make the dairy product unfit for human consumption. The bacteria responsible for yogurt or cottage cheese formation are *Lactobacillus bulgaricus* and *Streptococcus lactis*.

Steps

A. Clean a glass slide by washing it and then dipping it in acetone or 95% ethanol. Air dry. Do not touch the slide after cleaning. **Note:** this step is very important, unless you are using new slides, because bacteria are very tiny organisms which are easy to confuse with background dust and other contaminants.

B. Place a tiny sample of yogurt or cottage cheese on your microscope slide, using the end of a toothpick. Add a small drop of tap water and stir with the toothpick to spread out the cells. Add a coverslip and place on the microscope stage.

C. Focus the microscope on the slide using low power, and then switch to high power. If your microscope has an oil immersion lens, add a drop of immersion oil to the slide and view the bacteria under oil immersion.

1. The swarms of rod shaped organisms that you see are cells of *Lactobacillus*. *Streptococcus* bacteria are round shaped, and they might also be in your microscopic field of view. Can you see any structure within the cells of either type of bacteria? If so, what?

2. What organelle is found in both eukaryotic and prokaryotic cells?

3. Do you see any movement? If so, how would you describe it?

D. Repeat steps A and B above, but do not add a coverslip to the slide. Instead, allow the smear to dry completely.

E. Drip xylene on the slide for 2 - 3 minutes to extract any fat present in the smear. This is best accomplished by holding the slide at an angle and slowly running the chemical over the smear drop by drop using a medicine dropper or small pipet.

F. Drip 95% ethanol on the slide for 1 - 2 minutes to fix the bacteria in the smear.

G. Drip methylene blue solution on the slide for 1 minute.

H. Drop 95% ethanol on the slide for 1 minute, or until the slide is only faintly blue.

I. Rinse the slide by dropping tap water on it until the slide no longer stains the wash water.

J. Allow the slide to air dry and, without a coverslip, examine it under low and high power.

4. Does the methylene blue solution make a difference in the way you see the bacteria. If so, how?

K. Look at your own oral bacteria. Use a clean toothpick to scrape some of the tartar from between your teeth. Smear the scum on the microscope slide with the toothpick. Pass the slide over the small flame of an alcohol lamp or bunsen burner to "fix" the cells onto the slide.

L. Add a drop of crystal violet stain and wait 1 minute.

M. Drip tap water on the slide until the slide is only faintly purple, about 1 minute. Air dry the slide.

N. You may view this slide of bacteria from your mouth under the microscope, either with or without a coverslip. If you use a coverslip, add a drop of water to the slide before adding the coverslip.

5. The bacteria are tiny and darkly stained. They may be round, rod, or spiral shaped, and some may appear in clumps. Choose several different types noted on the slide, and draw the bacteria types that you see in the space provided below. Label them as to shape and grouping (if any).

O. View under both low and high power the three types of bacteria shown on the prepared slides.

6. Draw the three types in the space provided below and label them as to shape.

Procedure: Hour 2

Students need the following: *Paramecium*, *Euglena*, congo red stained yeast cells, methyl cellulose or protoslo, lens paper, and toothpicks.

The Kingdom *Protista*

The kingdom *Protista* contains microorganisms whose specialized intracellular organelles permit a far wider range of activities than the more primitive, prokaryotic *Monera*. *Protista* capabilities range from species which are animal-like hunters of food to plant-like photosynthesizers. Still others are fungus-like in that they feed on organic detritus. Although diverse, all *Protista* are unicellular eukaryotes.

There are four classes in the kingdom *Protista*, phylum *Protozoa*: *Sarcodina* (amoebae), *Ciliata* (ciliates), *Mastigophora* (flagellates), and *Sporozoa* (non-motile parasitic forms). You will observe during this hour a plant-like flagellate, the *Euglena*, and an animal-like ciliate, the *Paramecium*.

Steps

A. Place a small drop of the *Euglena* culture on a clean slide and add a drop of methyl cellulose, or protoslo. Stir the drop with a toothpick and add a coverslip.

B. View the slide under low power until you can identify the *Euglena*, and then switch to high power.

7. Approximately how large is your specimen? ______________________ μm.

8. Note the swimming action of *Euglena*. *Euglena* have two flagella and the one used for locomotion is visible under the microscope. Can you see the locomotive flagellum? ______________________

9. Look for the eyespot (stigma) and contractile vacuole near the base of the flagella. What color is the eyespot, and what is its function?

C. Note other parts of the *Euglena*. Near the center of the cell you will find a single nucleus. There are also chloroplasts. Each chloroplast may contain a single pyrenoid, which will appear as a clear, round area within the plastid. These pyrenoids **may be** associated with paramylon synthesis. Paramylon, the carbohydrate storage product unique to these microorganisms, appear as many refractive granules of various shapes and sizes within the cytoplasm.

10. Draw a *Euglena* in the space provided below, labeling all parts.

D. Note the numerous, helically arranged striations which appear near the surface of *Euglena*, immediately beneath the plasma membrane. The plasma membrane and the striations together constitute the pellicle.

11. Why is the pellicle not considered to be a cell wall? ______________________

12. Phototaxis is a directional movement in response to light. Note the movement of the *Euglena* in response to the light of your microscope. Are *Euglena* negatively (moving away from light) or positively (moving toward light) phototactic? ______________________

13. What is the adaptive significance of this behavior? ______________________

E. Place a small drop of the *Paramecium* culture on a clean slide and add a drop of methyl cellulose, or protoslo. Add a minute amount of congo red dyed yeast cells by using a toothpick. Gently mix the drop with the toothpick and add a coverslip.

F. View the slide under low power until you can identify the *Paramecium's* gross structures, and then switch to high power.

14. Approximately how large is the specimen? ______________________ µm.

G. As the *Paramecium* rotates, identify the oral groove. Cilia lining the groove create currents which sweep food (in this case congo red dyed yeast cells) into the gullet, where food vacuoles are formed. Find a *Paramecium* which has ingested some yeast cells.

15. Where are the yeast cells found inside the *Paramecium*? ______________________

16. Focus with the high power objective of your microscope on one yeast cell being digested. What color are the yeast cells when they are inside the *Paramecium*? ______________________

17. What factor might be affecting the color of the dye? ______________________

H. Use the high power objective to look closely at the contractile vacuole. You should see its radiating channels collect and then transfer excess water to the vacuole, which then drops the water out of the *Paramecium* through a modified area on the pellicle, or cell covering.

18. Do you see beating cilia on the pellicle of the *Paramecium*? ______________________

19. As the *Paramecium* becomes irritated by heat from the microscope light, or by the weight of the coverslip as its "pond" evaporates, it may discharge trichocysts. These will appear as fuzzy threads just on the outside of the pellicle. Are *Paramecia* positively or negatively phototactic? Why?

Procedure: Hour 3

Students need all listed equipment, as well as the following materials: leaves, razor blade, lens paper, sterile lancets, cotton balls, 70% alcohol, microscope slides and coverslips, and prepared slides of the human intestine and the cross section of a leaf.

The kingdoms *Plantae* and *Animalia* are comprised of multicellular plants and animals. Plants primarily photosynthesize for energy, and animals feed off plants and other animals. Their cells are interdependent and specialized to perform certain tasks on behalf of the entire organism.

You will observe examples of cells as they function in both plants and animals in this laboratory hour.

Steps

A. Slice a very thin cross section off a small sample of the leaves provided by the instructor. Carefully lay the slide on a microscope slide then cover with a drop of water and a coverslip.

B. Examine the leaf section first under the low power objective and then under the high power objective. If the cross section is not thin enough to see individual cells, step A should be repeated. Focus on a clear and orderly group of cells (a cell group which has not been disturbed by slicing during slide preparation).

20. How many different types of cells do you see? ____________________

21. Choose a representative cell from each cell type in your field of vision, and draw each cell in the space provided below. Label all cell structures you are able to identify.

22. Do all the cell types possess chloroplasts? If not, where do those cells which do not have chloroplasts obtain their energy to live?

____

____

____

____

C. Observe the prepared slide of a cross section of a leaf under both the low and high power objectives.

23. Does the prepared slide differ from your own slide of a leaf cross section? How? ____________

____

____

____

____

D. Sterilize the end of your ring finger by wiping it with cotton soaked in alcohol. Prick your finger with a sterile lancet. Place a drop of your blood on a clean slide and cover with a cover slip. Wipe your finger again with the alcohol soaked cotton to prevent infection.

E. View the slide of your blood under low and then high power objectives.

24. How many types of blood cells do you see? ____________________________

25. Choose a representative cell from each of the cell types you see, and draw each type in the space provided below. Label all cell structures that you observe.

26. What cell structure is missing in the red blood cells but is possessed by the white blood cells? ________

27. How can red blood cells reproduce without this vital cell organelle? ____________________
____
____
____
____
____

F. Observe the prepared slide of the human intestine under both low and high power objectives.

28. How many cell types do you observe? ____________________________

29. Choose a representative cell from each cell type that you observe in the prepared slide, and draw each cell type in the space provided below. Label all cell structures that you observe.

30. Are the intestinal cells similar to human blood cells? In what ways are they alike or different?

31. Are the intestinal cells and blood cells more like each other, or is one or the other more similar to a typical plant cell, such as the cross section of the leaf you observed earlier?

32. Could any of these cells from multicellular organisms be independent of their surrounding organizational support structure, such as the cells you observed during hours one and two of this laboratory? Why or why not?

Resources

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Terminology

Students should understand the following terms and concepts prior to taking the unit review.

Animalia
cell
Ciliata
compound microscope
electron microscope
eukaryotic
Mastigophora
Monera
Plantae
paramylon
phototaxis
polysomes
prokaryotic
Protista
Sarcodina
Schizomycetaceae
Sporozoa

Review
12. Cells

Name______________________________

Date______________________________

True or false

______ 1. Phototaxis is a reaction to
a) light
b) heat
c) cold
d) acid

______ 2. Compared to eukaryote cells, prokaryote cells
a) lack DNA
b) have distinct nuclei
c) lack membranous organelles
d) are much larger

______ 3. Which of the following organelles is found in all prokaryotic cells?
a) Golgi complex
b) ribosomes
c) mitochondria
d) centrioles

______ 4. What microscope uses beams emitted by a filament to form enlarged images of specimens?
a) compound
b) light
c) electron
d) dissecting

______ 5. Which organelle do plants possess but not animals?
a) chloroplasts
b) leucoplasts
c) cell membranes
d) nuclei

______ 6. The *Sarcodina* are in the kingdom
a) *Animalia*
b) *Monera*
c) *Plantae*
d) *Protista*

______ 7. *Paramecium* is a
a) flagellate
b) ciliate
c) pseudopod
d) sporozoid

______ 8. *Euglena* store their food in
a) starch grains
b) Golgi bodies
c) paramylon bodies
d) lipids

______ 9. The fermentation of dairy products is an ancient method of
a) preservation
b) spoilage
c) worship
d) producing *Sarcodina*

______ 10. *Schizomycetaceae* means
a) split personality
b) fission animal
c) small body
d) fission fungi

Short answer

1. How are bacteria classified? ____________________

2. How are the *Protista* classified? ____________________

Answers Found: p 114 - #4; p 115 - #2, 3, 9, & 10; p 118 - #5, 6, 7, & 8; p 119 - #1; Essay 1: p 115; Essay 2: p 118.

Diversity of Life and Reproduction

13. Laboratory

Bacterial Staining Procedures

When you have completed this laboratory you should be able to:

1. Demonstrate proper staining techniques for bacteria on solid or liquid media.
2. Demonstrate simple staining, gram staining, spore staining, and acid fast staining of bacteria.

Purpose

Bacteria are stained to demonstrate the morphological characteristics of the organism and to show the presence and location of biochemical constituents. No single stain will demonstrate all the known elements of the bacterial cell. As a result, special staining techniques have been developed for such parts as the capsule, cell wall, spores, and flagella. Still different staining techniques are commonly used for components such as fats, carbohydrates, and nucleic acids, and the application of histochemical techniques is continually expanding. For purposes of introduction, only four types of stains are described in this laboratory — the simple, the gram, the spore, and the acid fast stain.

This laboratory is a procedural exercise designed to introduce the student to techniques employed in microbiology laboratories. The student also will find that these procedures are used in subsequent laboratories in this manual.

Pre-lab

Supplies needed:

Equipment

- microscope.
- slides
- cover slips
- staining bar (optional)
- bunsen burner or other heat source
- inoculating loop
- eye dropper

400 ml. beaker and ring stand, or any other set up to make a boiling water bath.

Materials

- methylene blue
- 95% ethanol
- safranin
- acid alcohol
- water
- crystal violet
- Gram's iodine
- carbol fuchsin
- microscope immersion oil
- agar on petri plate, contaminated, or any bacterial growth source

Special Preparations

1) Methyl blue: add 1.48 g. of the dye to 100 ml. 95% ethyl alcohol. Let this stock solution stand covered for at least two days before use. Before using, dilute the stock solution. For each 10 ml. stock solution add 90 ml. distilled water. Dispense in dropper bottles.

2) Safranin: add 3.41 g. of the dye to 100 ml. 95% ethyl alcohol. Let this stock solution stand covered for at least two days before use. Before using, dilute the stock solution. For each 10 ml. stock solution add 90 ml. distilled water. Dispense in dropper bottles.

3) Crystal violet: add 13.9 g. of the dye to 100 ml. 95% ethyl alcohol. Let stand for two days, stirring frequently. Filter and store until needed. Before using, dilute the stock solution. For each 5 ml. stock solution add 5 ml. 95% ethyl alcohol and 40 ml. 1% aqueous solution of ammonium oxalate. Dispense in dropper bottles.

4) Carbol fuchsin: add 8.2 g. basic fuchsin to 100 ml. 95% ethyl alcohol. Let stand for two days, stirring frequently. Filter after two days and store. Prepare carbol fuchsin from this stock solution. Combine 1 g. basic fuchsin solution in 10 ml. 95% alcohol and mix with 5 g. phenol and 100 ml. distilled water. Dispense in dropper bottles.

5) Gram's iodine: mix together 2 g. potassium iodide and 1 g. iodine in 240 ml. distilled water and 60 ml. 5% aqueous solution of sodium bicarbonate. Dispense in dropper bottles.

6) Acid alcohol: add 1 ml. of 1% hydrochloric acid to 90 ml. 70% ethyl alcohol. Dispense in dropper bottles. **Note:** acid alcohol also is excellent for cleaning slides and coverslips.

Time Required:

The staining procedures laboratory requires three classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the listed equipment, as well as the following materials: sample bacterial growth on agar petri plate and methylene blue stain.

Aseptic Technique

Aseptic, or sterile, technique is used to manipulate microorganisms without introducing contaminants from the environment. Many of the problems encountered in microbiological laboratory procedure are due to contamination. Microorganisms exist everywhere, on the surface of all objects, and in the air. A conscious effort must be made to keep them out of a sterile environment. Here are some important precautions to take during all microbiological procedures.

1) Use only sterilized glassware or disposable plastic ware.

2) Wipe the work area with 70% alcohol before starting. Also rinse your hands with alcohol (use hand lotion at the end of the laboratory to counteract the dryng effects of the alcohol).

3) Be careful not to talk, sing, or whistle when performing these procedures. Your breath can create air currents which carry contaminants into the culture.

4) Never uncover any sterile surface until the moment before it is to be used. Return the cover as soon as you are finished. Never leave it open to the environment.

5) When removing the cap from a bottle, flask, test tube, etc., angle the container as far as possible to reduce the surface of the opening into which microorganisms can fall. **Do not hold the opening straight up into the air.**

6) When transferring bacterial cells use the following procedure:

a) Introduce the cells (inoculum) into a sterilized medium by transferring with an inoculating loop or needle. Heat the inoculating loop or needle to redness by flaming immediately **before** and **after** making the transfer. This flaming destroys any living forms on the surface of the loop or needle.

b) Grasp and hold the cap or cotton plug of the bottle, flask, or test tube with your little finger. **Do not place the cap or cotton plug on the laboratory bench.**

c) Immediately flame the mouths of the tubes from which cultures are taken and into which they are transferred, both **before** and **after** transfer. In addition to destroying any organisms on the lip of the tube, flaming tends to create outward convection currents, thus decreasing the chance of contamination.

(d) If you are transferring cells from petri plates, you cannot flame the lips of the plates. Instead, you hold the lid of the plate at an angle over the plate to avoid air-borne contamination. This technique is illustrated as follows in figure one.

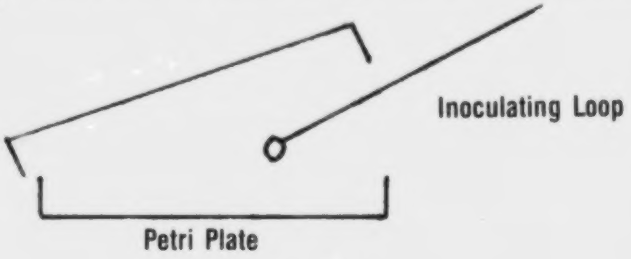

Figure 1

Solid Media Procedure

Bacteria found growing naturally may be cultured on solid media in the laboratory. Bacteria on solid media grow from a few cells to a mass of daughter cells which can become visible to the naked eye. Such a cell cluster is called a colony.

The surface of colonies may be classified as smooth, rough, dry, moist, or pigmented. The following diagrams represent typical bacterial colony shapes. You will find these descriptions and diagrams useful in gross bacterial classification.

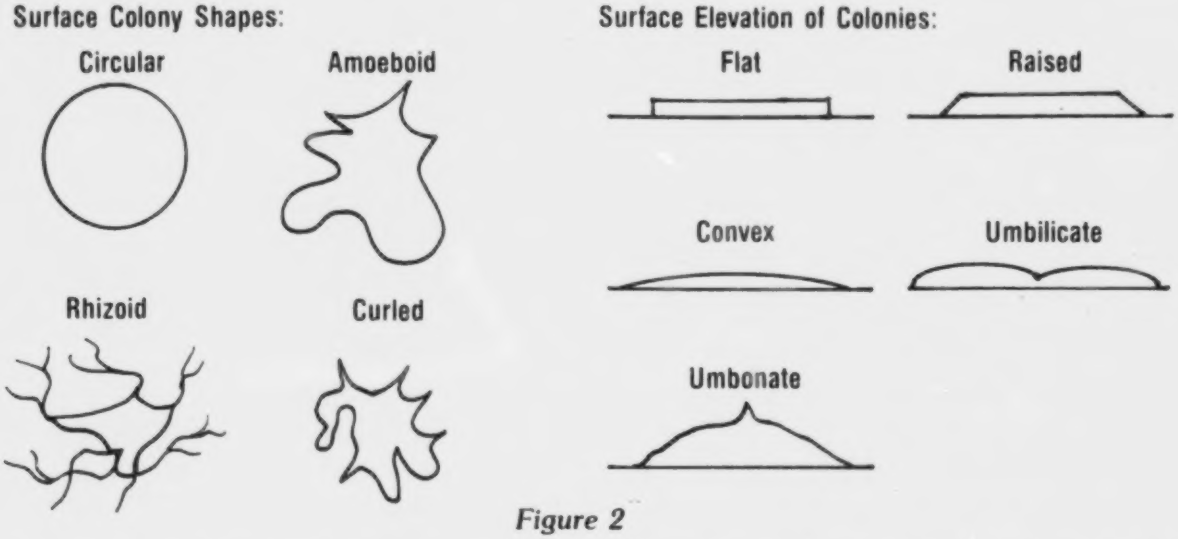

Figure 2

An easy way to make a solid medium is to add a solidifying agent to a broth medium which will harden as it cools. The broth contains nutrients dissolved in water, with the mix varied according to the bacteria to be cultured. Usually the solidifying agent is agar, a substance obtained from marine algae and available in dried, purified form. Although different agars vary widely in their physical properties, the usual melting point is 97 – 100° C. Thus agar may be liquified for use by boiling. On cooling, media containing agar solidify at about 42° C. If they are to be inoculated before hardening, they are usually cooled to 45 – 47° C., a temperature that is not harmful to most bacteria for short periods of time. Once solidified, agar media may be incubated over the entire range of temperatures up to 70° C. without melting.

Besides petri plates, the most commonly used agar medium, agar slants and stabs are two standard solid media methods for culturing bacteria. An agar stab is simply broth and agar which have been hardened in a test tube. The tube is capped with a loose plastic cap or cotton plug. An agar slant also is hardened in a test tube, but the mixture is allowed to harden on a slant so it has the maximum surface area exposed inside the test tube. It is inoculated with an inoculating loop and bacteria and is capped in a manner similar to an agar stab. It is not as easy to see typical colonial growth on slants and stabs as with bacteria grown on petri plates, but they are convenient and space saving methods for maintaining stock cultures.

Agar is a galactan, a complex carbohydrate composed of galactose molecules which are not subject to breakdown by most bacteria. It is not a food source, and therefore the medium must contain a nutrient supply for the bacteria. Other hardening agents used for special purposes include gelatin and silica gel.

The procedure for transferring bacteria grown on solid media is as follows:

1) Place a small drop of tap water on a clean glass slide. Using aseptic technique, remove a sample of organisms from the colony and emulsify in the drop of water until the drop is faintly turbid. Spread over an area approximately the size of a dime and allow to air dry.

2) When the smear is completely dry, heat fix it by passing the slide quickly, smear side up, through a flame two or three times. The slide should feel barely warm to the back of your hand. Excessive heating will distort the organisms.

Liquid Media Procedure

Bacteria also can be found in nature in liquid media. In the laboratory bacteria can be grown in a test tube in a liquid broth medium. There are many recipes for good broths, depending on the type of bacteria to be grown. All liquid media must provide the proper physical and chemical environment and nutrient substances in water solution.

Broth solutions may be affected in different ways by the growth of bacteria. You will find these descriptions convenient when attempting to describe and/or classify bacteria.

1) Turbidity: a cloudiness, more or less dense.

2) Pellicle formation: a small mass of cells floating on top of the broth.

3) Sediment: a deposit of cells resting at the bottom of the broth culture which will swirl up if the tube is tapped gently.

The procedure for transferring bacteria grown in liquid media is as follows:

1) Place a loopful of liquid containing the organism on a clean glass slide, using aseptic technique. Spread over a dime sized area and allow to air dry.

2) When the smear is completely dry, heat fix it by passing the slide quickly, smear side up, through a flame two or three times. The slide should feel barely warm to the back of your hand. Excessive heating will distort the organisms.

Simple Staining

Simple staining is possible because of the chemical differences which exist between bacterial cells and their surroundings. Cells will absorb a selected stain differently from their medium and the contrast between the cells and their environment can be shown. Methylene blue is one such simple and basic dye, formula $C_{16}H_{18}C1N_3S \cdot 3H_2O$, which is especially useful for staining bacteria and nuclear material from eukaryotic cells. Methylene blue also is an oxidation reduction indicator, because it has two forms, a blue form and a colorless form, with the colorless form appearing in acidic mixtures. Incidentally, methylene blue also is used as an antidote for cyanide poisoning.

In this laboratory hour you will stain your own saliva (a personal bacterial source in liquid medium) with methylene blue.

Steps

A. Prepare a smear of your spit on a clean glass slide. **Do not stick a hot inoculating loop in your mouth. Let the loop air cool before placing in your mouth to collect saliva.** Air dry and fix with heat, following the instructions given earlier.

B. Apply a drop of methylene blue stain to the slide smear. Let sit for a period of two minutes.

C. Gently wash off stain with droppers of tap water, air dry the slide, and examine with the microscope under high power or under oil immersion.

1. Draw in the space provided below what you see on your slide.

2. How many different bacterial shapes do you see? ____________ Of the three basic bacterial shapes (discussed in laboratory twelve) which ones are living in your mouth? ____________

Cocci, or round bacteria, are further classified according to the way the cells divide. Some cocci divide in one plane only and form chains of cocci called *Streptococcus*. Some cocci divide in any plane and form irregular clumps of cells called *Staphlyococcus*. Some cocci divide in two and stick togethr; the double cells are called *Diplococcus*. Some cocci divide in three planes at right angles to each other forming packets of eight; these are called *Sarcina*.

3. Do you see any cell groupings on your stained slide? You may well see groupings that have not been described above. What are these?

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D. Your instructor will give you a contaminated petri plate. Make a smear slide from this petri plate, using aseptic technique and the procedures for bacteria grown on solid medium.

4. Draw in the space provided below what you see on this slide.

Procedure: Hour 2

Students need all listed equipment, and the following materials: crystal violet, Gram's iodine, 95% ethanol, and safranin.

The Gram Stain

The gram stain is a type of differential stain, a staining procedure based on the physical and chemical differences which exist between species of bacteria, in additon to the cell-medium difference exploited in simple staining. Differential stains require two or more dyes to develop the contrasts being sought.

The gram stain was named for its developer, Christian Gram, who used it while working on the etiology of respiratory diseases at the Municipal Hospital in Berlin in 1883 — 1884. The gram staining procedure today is used by bacteriologists more than any other staining technique. It is indispensible for the identification of unknown organisms, and it is of great importance in the diagnosis of infectious diseases such as gonorrhea, meningitis, and pneumonia.

The gram stain requires four different solutions: a basic dye, a mordant, a decolorizing agent, and a counter-stain.

Dyes

Dyes may be divided into two groups, basic and acidic. If the color resides in the positive ion of the dye, it is called a basic dye, or stain. Methylene blue and crystal violet are both basic dyes. If the color resides in the negatively charged ion, it is called an acidic dye, or stain.

Mordants

A mordant is a substance which increases the affinity or attraction between the cell and the dye. It helps fix, or, more strongly stain the dye on the cell. Examples of mordants are acids, bases, metallic salts, and iodine. A mordant also makes it more difficult to wash the dye out of the cell after staining. Iodine acts as the mordant in the gram staining procedure.

Decolorizers

A decolorizing agent is a substance which removes the dye from a stained cell. Some stained cells decolorize more easily than others. The gram stain capitalizes on this phenomenon by taking advantage of variations in the rate of decolorization in bacterial cells. Ninety-five percent ethanol is used as the decolorizing agent in this laboratory exercise.

Bacterial cell walls which tend to retain the dye in spite of the application of a decolorizing agent are called gram positive bacteria. Bacterial cell walls which lose the dye are called gram negative organisms. It is believed that the cell walls of the two types of bacteria are structured differently, the gram positive bacteria having thicker cell walls but the gram negative bacteria having an additional outer covering of some material chemically different from any thing found in the gram positive organism.

Counter-stains

The counter-stain is a basic dye of a different color from the initial dye. The purpose of the counter-stain is to give the decolorized cells a color different from that of the initial stain. Those organisms that are not readily decolorized retain the color of the initial basic dye, and those that are readily decolorized take the color of the counter-stain. Safranin will be used as the counter-stain in this laboratory exercise.

You will practice the gram staining procedure on your own oral bacteria.

Steps

A. Prepare a smear of your spit on a clean glass slide, air dry, and fix with heat following the instructions on aseptic technique given earlier in this laboratory. **Do not stick a hot inoculating loop in your mouth. Let it air cool before using.**

B. Apply crystal violet to the heat fixed smear for exactly one minute. **Timing is very important.**

C. Wash with water, drop by drop, until colored water no longer runs off the stained slide.

D. Rinse with Gram's iodine solution. Cover with Gram's iodine solution for exactly one minute.

E. Wash with water, drop by drop, until colored water no longer runs off the stained slide.

F. Add 95% ethanol slowly, drop by drop, to the tilted slide until no more purple dye washes off. This should take **exactly** 30 seconds.

G. Counter-stain with safranin for 30 to 60 seconds.

H. Wash with water, drop by drop, until colored water no longer runs off the slide. Air dry the slide, and observe it under the microscope on high power or under oil immersion. Show your slide to your instructor.

5. Draw in the space provided below what you see on your slide. Color bacteria blue if they are gram positive, and pink if they are gram negative.

6. What would happen if cells were first stained with crystal violet, then treated with Gram's iodine, and then, without decolorization, exposed to safranin?

7. Can you tentatively identify any of the bacterial organisms in your spit? If so, what might they be?

Procedure: Hour 3

Students need all the equipment listed, and the following materials: carbol fuchsin, boiling water bath, 95% ethanol, methylene blue, and acid alcohol.

During this hour you will do both the spore stain and the acid fast stain. Both are differential staining procedures.

The Spore Stain

The spore stain demonstrates the presence of bacterial spores. Among eubacteria (true bacteria) the ability to form endospores is a relatively rare ability. Endospore formation adds considerably to the survival abilities of bacteria, and those with the ability to form endospores must be considered to be a special physiological group among the eubacteria.

All known spore formers are rod shaped bacilli, with the exception of *Sporosarcina ureae*, which is a coccus. Most are motile and gram positive, although as a colony ages they can become gram negative or gram variable. The spores are most simply observed as intracellular refractile bodies in unstained cells, or as colorless areas in cells fixed with a simple stain.

Some spore formers are pathogenic for insects or for higher animals, and their characteristic habitat is the soil. There are three genera of spore formers, the *Sporosarcina* which are aerobes, the *Bacillus* which are both strict aerobes and facultative anaerobes, and the *Clostridium* which are strict anaerobes. Many *Bacilli* produce disease in man and animals, such as *B. anthracis*, the causative agent of anthrax. Some *Clostridia*, such as *C. tetani*, are the principal agents of wound infection. Others are powerful toxin producers in canned foods.

The spore wall of such bacteria is relatively impermeable to staining, but dyes can be made to penetrate it by heating the preparation. The same impermeability then serves to prevent decolorization of the spore through such procedures as the alcohol treatment employed during hour two. Other structures are then counter-stained for easy identification. Spores are commonly stained with carbol fuchsin.

The Acid Fast Stain

The earliest differential stain was a procedure developed by Robert Koch during his original work on tuberculosis in 1881. He used what is now termed the acid fast stain to differentiate between *Mycobacterium*, the bacterium which causes human tuberculosis, and the tissue cells and other microorganisms taken as samples from tubercular patients.

The acid fast stain, also known as the Ziel-Neelsen procedure, is of great diagnostic value in the detection and identification of mycobacteria. Mycobacteria means "fungus-bacteria", and they are a special group of bacteria which grow in a rudimentary fungus-like fashion (in strands of cells) before breaking up into irregular, often branched rods. The genus *Mycobacterium* contains several species that are pathogenic to man and other vertebrates, such as *Mycobacterium tuberculosis*. There are also many nonpathogenic soil dwelling species.

Mycobacterial cells contain waxy materials which make them difficult to stain with basic aniline dyes, such as carbol fuchsin. Mycobacteria do take a stain if it is applied as a hot solution, but once the stain has penetrated the cell walls, it is extremely difficult to remove. In fact, mycobacteria even resist destaining with a mixture of hydrochloric acid and alcohol. The cells of other bacteria are readily destained with this strong mixture.

In the acid fast stain procdure, a smear of cells on a slide is flooded with carbol fuchsin and heated on a steam bath. Following this, decolorization with acid alcohol is carried out, and, finally, a contrasting (blue or green) counter-stain is applied. Acid fast bacteria appear red, while all other bacteria take on the color of the counter-stain.

Further Precautions For the Acid Fast Staining Procedure

Certain precautions should be taken in the preparation of smears to be examined for acid fast organisms.

1) Use only new, clean slides to avoid sample contamination by acid fast bacteria that may be found on used slides from previous positive stains.

2) Use distilled water and reagents free of acid fast saprophytes and debris.

3) Place smears of a uniform, medium thickness on the slide.

4) Separate slides on staining bars, and add the stain with a dropper, being careful not to touch the slide with the tip of the dropper. The stain should be fresh and deep red in color.

5) Do not allow the stain to dry on the slide; add stain as needed to maintain the film.

6) If a free flame is used in heating the stain, great care must be taken to avoid boiling.

7) Do not use a blotter to dry the slide. Acid fast bacteria may be present on blotting tissue and would be transferred to the slide.

8) Be certain that the lens of the microscope is clean and free of acid fast material from slides previously examined.

Steps

A. Prepare two smears of your spit on two clean glass slides, air dry, and fix with heat following the instructions on aseptic technique given earlier in this laboratory. **Do not stick a hot inoculating loop in your mouth. Let it air cool before using.**

B. Place the slides on staining bars over a cup of boiling water and flood both smears with carbol fuchsin. Keep the slides well flooded with stain and steam for at least 7 minutes. **This can be messy, so be careful.** The stains should be fresh and deep red in color.

C. Thoroughly rinse off all excess stain in tap water.

D. Test one slide for spores: **Remember, timing is critical.**

1) Flood the slide with alcohol for 10 to 20 seconds and immediately rinse in water.

2) Counter-stain for 30 seconds with methylene blue; wash, dry, and examine.

8. What color are the vegetative structures in your slide? ______________________________

9. What color should the spores be in your slide? ______________________________

10. Draw some typical cells as they appear on your slide in the space provided below. Note their grouping and also their stained color.

11. If there were spore forming bacteria in your saliva, what might this signify? ____________________

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E. Test the other slide for acid fast bacteria.

1) Flush **all** freely removeable stain from the entire slide with acid alcohol.

2) Flood the entire smear with acid alcohol and allow it to stand for **at least** 10 minutes. It is impossible to over destain *Mycobacterium tuberculosis*.

3) Wash with water.

4) Counter-stain for 1 to 3 minutes with methylene blue.

5) Wash with water.

6) Let this slide dry thoroughly and examine under high power or with an oil immerison microscope.

12. Draw some typical cells as they appear on your slide in the space provided below. Note their grouping and also their stained color.

13. Why should acid fast organisms be difficult to stain with ordinary methods? ____________________

____

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14. Why is it necessary to decolorize with acid alcohol? Explain. ______________________________

____

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____

15. Did you find acid fast bacteria in your saliva? ____________ If you did, name two possible sources for the presence of the acid fast bacteria. ____

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____

____

Resources

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Volk, W. A. *Essentials of Medical Microbiology*. 2nd Ed. J. B. Lippincott: Philadelphia, 1982.

Wiese, C. R. "Archaebacteria," *Scientific American*, June 1981.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

acid fast stain
agar
aseptic technique
cocci
colony
counter-stain
decolorizer
differential staining
dye
gram stain
gram negative
gram positive
liquid media
mordant
mycobacteria
pellicle formation
sediment
simple stain
solid media
spore stain
turbidity

Review

13. Bacterial Staining Procedures

Name ____________________

Date ____________________

Multiple choice

_____ 1. The reagent in gram staining which acts as a mordant is

- a) crystal violet
- b) Gram's iodine
- c) alcohol
- d) safranin

_____ 2. The counter-stain used in Ziel-Neelsen acid fast staining is

- a) crystal violet
- b) methylene blue
- c) safranin
- d) none of these

_____ 3. Which reagent must be timed most carefully in gram staining?

- a) crystal violet
- b) acid alcohol
- c) methylene blue
- d) potassium permanganate

_____ 4. Decolorization in the acid fast staining procedure is accomplished by

- a) alcohol
- b) water
- c) potassium permanganate
- d) safranin

_____ 5. The proper sequence of staining in gram staining is

- a) crystal violet, alcohol, Gram's iodine, safranin
- b) crystal violet, safranin, alcohol, Gram's iodine
- c) crystal violet, Gram's iodine, alcohol, safranin
- d) none of these

Short answer

6. What will happen to a smear if it is not heated before staining? ____________________

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7. Give an example of simple staining as compared to differential staining. ____________________

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8. Some bacteria are said to be gram variable. What would be the staining characteristics of such organisms?

9. Give two reasons why agar must be cooled before inoculating and pouring

10. Tell how the planes of division of cocci can be a clue to their classification.

Answers Found: p 127 - #9; p 128 - #6; p 129 - #10; p 130 - #1 & 7; p 131 - #3 & 5; p 132 - #8; p 133 #2 & 4.

14. Laboratory

Isolation of Pure Cultures from Mixtures of Bacteria

When you have completed this laboratory you should be able to:

1. Observe the morphology and motility of bacteria.
2. Isolate pure cultures from mixtures of bacteria.
3. Make a presumptive verification of a pure culture isolate.
4. Inoculate stock cultures of bacteria.

Pure Culture Techniques

In order to study the properties of a bacterial species, it is necessary to work with that species in a pure culture, that is, in a culture free of all other types of bacteria. To do this, a single cell often is isolated from other cells and cultivated so that its offspring remain isolated. This is called pure culture technique.

Robert Koch

Less than one hundred years ago scientists thought that bacteria had the capacity to change their shape and size, and perhaps even function, since bacteria were found naturally in mixed populations. Robert Koch, a German physician, demonstrated the error of this thinking through developing pure culture techniques. Koch combined a medical practice with an extremely productive research career, for which he received the Nobel Prize in 1905. Koch's award resulted from his interest in studying bacteria which cause certain diseases. He recognized that it was necessary to have simple methods for obtaining pure cultures of target bacteria. Koch concluded that the isolation of a pure culture required a solid medium on which an isolated single cell could multiply into a colony.

Koch initially experimented with growing bacteria on the cut surfaces of potatoes, but he found that a lack of particular nutrients prevented growth of some bacteria. Koch then decided that it would be advantageous to solidify a liquid nutrient designed for the growth of a particular bacteria. He eventually hit upon agar as the best hardening agent. His was the same procedure as that used today and described in laboratory thirteen.

Bacterial Isolation

Koch perfected two methods for isolating bacteria on agar. The first is the streaking method, in which an inoculating loop of bacteria is lightly streaked over hardened agar and then incubated. The other is the pour plate method, in which bacteria are added to agar just before it hardens and then poured into a dish where the bacteria incubate. Koch's laboratory also designed the petri plate, a two part glass dish which can be readily sterilized. It is named after the technician who developed it and remains unchanged to this day.

There are many methods for obtaining pure cultures of bacteria. This laboratory is designed to duplicate some of Robert Koch's original experiments in bacterial isolation. Students will also observe and record some of the distinguishing biochemical activities of the bacteria thus isolated.

Pre-lab

Supplies needed:

Equipment

microscope
inoculating loop
2 sterile petri plates
bunsen burner or open flame source
slides
incubator
test tube rack
inoculating needle

Materials

suspension containing two organisms
2 TSY or nutrient agar plates
2 TSY or nutrient agar deeps
2 TSY or nutrient agar slants
2 sterile blanks
2 gelatine deeps
2 nitrate broths
2 lactose ferments
2 sucrose ferments
Kovac's reagent
crystal violet
Gram's iodine
ethyl alcohol
safranin
2 tryptone broths
2 glucose ferments
2 maltose ferments
nitrite test regents "A" and "B"
immersion oil (optional)

Special Preparations

1) Set the incubator at 37° C.

2) Make the crystal violet, Gram's iodine, and safranin according to the directions in laboratory thirteen.

3) Kovac's reagent (also known as the indole test): mix 75.0 ml. n-Amyl alcohol with 25.0 ml. hydrochloric acid and 5.0 grams p-dimethylamine benzaldehyde.

4) Nitrite test reagents.

Solution A: dissolve 8 grams sulfanilic acid in 1 liter 5 Normal acetic acid (1 part glacial acetic acid to 2.5 parts water).

Solution B: dissolve 5 grams dimethyl-alpha-naphthylamine in 1 liter 5 Normal acetic acid.

Do not mix solutions.

5) All the following broths, ferments, blanks, and agar, as well as glassware containing them **must** be sterile. If available, an autoclave is a very useful instrument for sterilization, since moist heat is a more efficient killing agent than dry heat, and an autoclave uses supersaturated steam at a temperature of 121° C. Autoclave (or pressure cook) all materials and glassware for 30 minutes at 121° C. and 15 pounds per square inch pressure. This work should be done in advance of the actual laboratory, and, after cooling, the various test tubes and petri dishes may be refrigerated until the actual laboratory session.

6) Ferments: dissolve 40 grams of each sugar — glucose, lactose, maltose, and sucrose — in 1 liter each distilled water. Add ten drops phenol red indicator (phenolsulfonphthalein) to each sugar-water mixture. Phenol red is suitable for use as a pH indicator, since at pH 6.8 it is yellow and at pH 8.2 it is red. Fill test tubes with 25 ml. of each sugar solution. Make one test tube of each type of sugar for each laboratory group. Label each test tube as to what type of sugar it contains. Add durham tubes to the test tubes, filling the tubes with the sugar solution. Plug with rolled cotton wads and sterilize.

7) Tryptone broth: make tubes of 1% tryptophane broth (such as Difco B123). Fill test tubes with 25 ml. of the solution. Make one test tube for each laboratory group. Label the test tubes. Plug with rolled cotton wads and sterilize. Refrigerate.

8) Nitrate broth (such as Difco B268): fill test tubes with 25 ml. of the solution. Make one test tube for each laboratory group. Label the test tubes. Plug with rolled cotton wads and sterilize. Refrigerate.

9) Sterile blanks: fill test tubes with 25 ml. distilled water. Make one test tube for each laboratory group. Label the test tubes. Plug with rolled cotton wads and sterilize. Refrigerate.

10) Nutrient agar, or TSY (trypticase-soya-yeast) agar: either agar mixture may be used. Soften in distilled water according to package directions, and heat, stirring, until dissolved. The exact proportions of agar to water vary depending on the type of agar. Fill test tubes with 25 ml. agar solution. Make four test tubes for each laboratory group. Label the test tubes. Plug with rolled cotton wads and sterilize. Cool two of every four test tubes of agar on a slant (these are known as agar slants; the other two are known as agar deeps), and when they have hardened, refrigerate all four test tubes.

11) Also make two agar petri plates per each laboratory group, using the agar preparation directions given in #10 above. Fill each plate approximately half full of nutrient or TSY agar. Sterilize and allow the agar to harden. Transfer the agar petri plates to a refrigerator, and store in an inverted position until the laboratory. They are to be inverted so that moisture will not condense on the lids and drip into the agar.

12) Gelatin deeps: dissolve nutrient broth in distilled water to which has been added 12 to 15% gelatin. Fill test tubes with 25 ml. of the solution. Make one test tube for each laboratory group. Label the test tubes. Plug with rolled cotton wads and sterilize. Refrigerate.

13) Suspension containing two organisms: each student group should receive a different suspension containing two organisms unknown to them. These unknowns should be prepared, labeled by number, and incubated before the laboratory sessions. The instructor may wish to conduct this laboratory in one of two ways.

If the instructor wishes the suspension to contain bacteria with obvious colonial color differences, the suspension should contain two of the following bacteria, *Escherichia coli*, *Sarcina lutea*, and *Serratia marescens*. All are readily available from several suppliers.

If the instructor wishes the unknown bacteria not to have color clues, the suspension can be selected from the following bacteria, *Aerobacter aerogenes*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus albus*, or *Streptococcus zymogenes*. Several of these are available from commercial sources, and the rest are available from the American Type Culture Collection.

Time Required

The isolation of Pure Cultures from Mixtures of Bacteria laboratory requires five classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need all equipment and the following materials: a suspension containing two organisms, and two nutrient agar or TSY plates.

During this laboratory hour you will observe the morphology and motility of bacteria. You also will begin to isolate pure cultures from a mixture.

Steps

A. Gently mix the suspension of unknown bacteria by rolling the test tube in your hands. Prepare a wet mount of the suspension and examine under low and high power microscope objectives.

1. Describe the bacteria and their movement.

2. How is this "Brownian movement" different from true movement?

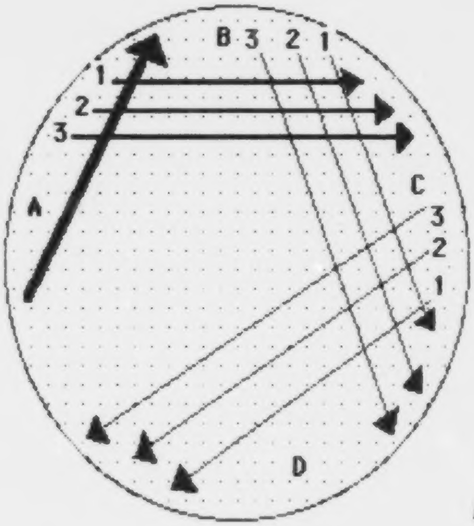
B. Make a gram stain of the suspension (per instructions in laboratory thirteen), and examine under the low and then the high power microscope objectives. Also examine under oil immersion, if your microscope is so equipped.

3. You should see two bacterial types. Describe them as to shape, observable planes of division, and whether they are gram positive or negative.

a) Bacteria: #1:

b) Bacteria #2:

C. Streak the suspension on each of the two agar plates provided. There are many ways to streak an agar petri plate, but figure one below shows a standard method for isolating individual colonies of bacteria.



A = first streak with loopful of bacterial suspension

B = resterilize loop, streak three times

C = resterilize loop, streak three times

D = resterilize loop, streak three times

Figure 1

D. Label your petri plates with the following information: unknown suspension number, date, and student names or group name. Place plates (inverted) in the 37° C. incubator. Incubate them for 24 to 48 hours.

Procedure: Hour 2

Students need all equipment and the following materials: the materials from procedure, hour one, and two agar deeps (the agar in the test tubes should be heated in a boiling water bath to melting point) and two sterile petri plates.

You will observe colonial morphology and continue the isolation of pure cultures during this laboratory hour.

Steps

A. Pour and cool two plates of agar.

B. While the agar plates are cooling, observe the colonies streaked during hour one of this laboratory. On the basis of your observations, select two well isolated colonies which appear to be representative of the two different organisms present. Make a gram stain of each to further determine that two different organisms are represented. Repeat the entire procedure, if necessary, until you are certain that each colony represents one and only one type of bacteria, and that the two colonies are composed of different bacteria. **If there are no isolated colonies, repeat procedure, hour one, before proceeding with this laboratory.**

4. Observe the two bacterial types. Describe them as to colonial color and shape, individual shape, observable planes of division, and whether they are gram positive or negative.

a) Bacteria #1: ____________________

b) Bacteria #2: ____________________

C. Streak two agar plates, placing samples from the two selected colonies on separate plates. Again, label the plates with the unknown suspension number, date, bacterial type (shape and gram stain type), and student names or group name. Incubate at 37° C. in an inverted position for 24 to 48 hours. **Note:** the original plates should be kept in reserve at room temperature until growth on the new plates is assured.

Procedure: Hour 3

Students need all equipment and the following materials: the materials from hour two, and two agar slants.

The student will make a presumptive verification of the pure cultures isolated, and inoculate a stock culture slant of both bacteria.

Steps

A. Make a gram stain of a well isolated colony from each of the two separate petri plates. **If there are no isolated colonies, or if there is more than one bacterial type in the colonies, pour another agar plate and repeat procedure, hour two.**

5. Observe the two bacterial types. Describe them as to colonial color and shape, individual shape, observable planes of division, and whether they are gram positive or negative. Label the petri plate which is (a) and that which is (b).

a) ____

____

____

____

b) ____

____

____

____

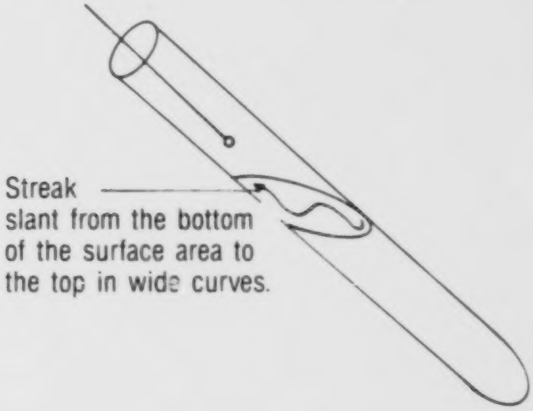
____

6. Attempt a presumptive verification of the genera of the two bacteria, based on your studies to date. If *Bergey's Manual* is available, check your presumptions with their classification system.

a) Bacteria #1: ________________________________

b) Bacteria #2: ________________________________

B. Transfer the **same colonies** that you have described and gram stained into two different agar slants.



Streak
slant from the bottom
of the surface area to
the top in wide curves.

Figure 2

C. Label the slants of the two bacteria with the suspension number, date, bacterial type (shape and gram stain type), and student names or group name. Incubate at 37° C. for 24 to 48 hours. The original plates should also be kept in the incubator, again as a reserve.

Procedure: Hour 4

Students need all equipment and the following materials: the materials from laboratory hour three, and two each of the following — sterile blanks, gelatin deeps, tryptone broths, nitrate broths, glucose ferments, lactose ferments, maltose ferments, and sucrose ferments.

Hour four procedures are designed to study some of the biochemical activities of each group's two isolated bacteria. If the bacteria ferment any of the sugars, that shows that the bacteria are using that sugar as a carbon source for part of their nutritional requirement. If the bacteria degrade the protein broths in any way, that shows that the bacteria are using that particular protein as a nitrogen source as part of their nutritional requirement.

Steps

A. Make a suspension from each of the stock cultures, and use the suspensions as inocula in the following tests. Make the suspensions by following aseptic procedures. Take a loopful of bacteria from each of the stock cultures, and swish it around in distilled water in a sterile blank.

B. Inoculate the tryptone broths, nitrate broths, glucose ferments, lactose ferments, maltose ferments, and sucrose ferments. There are to be two test tubes for each of the broths and ferments, with one of each type to be inoculated with separate bacteria. Put a loopful of a suspension in each test tube, using aseptic technique. Label each test tube. **Note:** confusing test tubes at this point will require a repeat of the procedures.

C. Make a gelatin stab of each bacteria by using an inoculating needle with bacteria taken from the stock agar slants. You literally will stab a needle into the middle portion of the gelatin test tubes. Follow aseptic procedure. Label both test tubes.

D. Incubate the gelatin cultures at room temperature for 24 to 48 hours. Incubate the broths and ferments at 37° C. for 24 to 48 hours.

7. Why must you incubate the gelatin stabs at room temperature? ____________________

____

____

____

____

____

Procedure: Hour 5

Students need all listed equipment as well as the following materials: the materials from procedure hour four, nitrite test reagents A and B, and Kovac's reagent.

Students now will read and record the biochemical activities of the unknowns in order to classify the bacteria.

Steps

A. Read and record the results of the sugar fermentations in the following manner.

1) Gas has been produced (positive test) if there is a large bubble in the durham tube.

2) Acid has been produced (positive test) if there is a pinkish color to the solution in the test tube.

3) Acid and gas may both be produced by the same organism.

4) No change has occurred if there is no color change and no gas formation. Bacterial growth is indicated by turbidity. If no growth has occurred, your test is not valid and must be repeated.

8. Record your results in figure three below.

Sugar Fermentation

Unknown	Gas	Acid	No Change	Gram	Shape
#1					
#2					

Figure 3

B. Test the nitrate broths for nitrate reduction to nitrite by adding 10 - 15 drops of first "A" and then "B" nitrite reagents. Do not touch the dropper against the mouth of the culture tubes. A pink or red color changing to brown will appear if nitrite ions are present.

9. Record your results in figure four below.

Nitrate Reduction

Unknown	Positive/Negative	Gram	Shape
#1			
#2			

Figure 4

10. What does the ability to reduce nitrates indicate about a bacterium?____________________________________

____

____

____

____

C. Test the tryptone broth for indol formation by adding 5 - 10 drops of Kovac's reagent. Do not touch the dropper against the mouth of the culture tube. A red color will appear in the supernatant alcohol layer if indol is present. Results from this test may take 5 - 10 minutes to occur. The tryptone broth contains the amino acid tryptophane, and some bacteria possess the ability to split off the side chain, leaving indol.

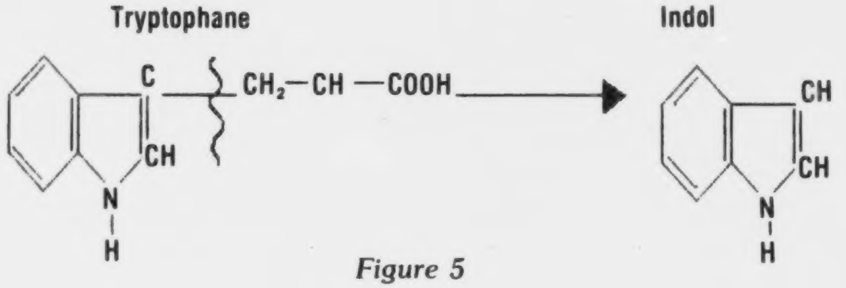


Figure 5

11. Record your results in figure six below.

Indol Formation

Unknown	Positive/Negative	Gram	Shape
#1			
#2			

Figure 6

12. What does the ability to form indol from tryptophane indicate about a bacterium?________________

____

____

____

D. Observe the gelatin deep for evidence of liquefaction. Cool the gelatin tube in cold water, if necessary, to attempt to solidify it. Hold until next period if no liquefaction is observed.

13. Record your results in figure seven below.

Gelatin Liquefaction

Unknown	Positive/Negative	Gram	Shape
#1			
#2			

Figure 7

14. What does the ability to liquefy gelatin indicate about a bacterium?________________

____

____

____

____

15. What other "tests" can you think of to determine biochemical differences in bacteria? What might these tests demonstrate?________________

____

____

____

____

16. At this point the instructor will provide a chart, taken from *Bergey's Manual* or similar guide, which delineates the biochemical and physical characteristics of those bacteria which might be the species in your unknown suspension. Identify the unknowns:

1) ____

2) ____

Resources

Berg, H. "How Bacteria Swim," *Scientific American*, August 1985.

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Blackemore, Richard P. and Richard B. Frankel. "Magnetic Navigation in Bacteria," *Scientific American*, December 1981.

Costerton, J. W., G. G. Geesey, and K. J. Cheng. "How Bacteria Stick," *Scientific American*, January 1978 (#1379).

Difco Manual, 9th Ed. Difco Laboratories: Detroit, 1977.

Hugh, R. and E. Leifson. "The Taxonomic Significance of Fermentative Versus Oxidative Metabolism of Carbohydrates by Various Gram-negative Bacteria," *Journal of Bacteriology*, Vol. 66.

Klyver, A. J. "Some Aspects of Nitrate Reduction," *Symposium on Microbial Metabolism*. Institute Superiore di Sanita: Rome, 1953.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

blank
Brownian movement
deep
ferment
pour plate method
pure culture
slant
streak method

Review

14. Isolation of Pure Cultures from Mixtures of Bacteria

Name______________________________

Date______________________________

Essay

1. Define the following terms

   a. blank ______________________________

   b. ferment ______________________________

   c. broth ______________________________

   d. deep ______________________________

   e. slant ______________________________

2. There are two bacterial types growing in a particular medium. One species can use maltose as its carbon source, while the other can not. The other can utilize only glucose. Each species has a characteristic appearance. How can one separate and identify the two types?______________________________

3. Design an experiment which would select for a nutritional mutant (a bacterial race which deviates nutritionally from others of its species).

Answers Found: pp 138, 139, & 143 - #1; Essays #2 & 3 - thought.

15. Laboratory

Isolation of Staphylococcus

When you have completed this laboratory you should be able to:

1. Observe some of the organisms which grow in the human throat.
2. Isolate a potential pathogen, *Staphylococcus*.
3. Determine the pathogen's antibiotic sensitivity pattern.

Parasitic Relationships

When only one member of a symbiotic relationship benefits from the association, the relationship is said to be parasitic. The organism which benefits from the relationship is the parasite, or pathogen, and the one which does not is the host. The host is usually harmed by the relationship and sometimes is killed by it. A pathogen usually is specific to the host it attacks and often also to the environment in which it may become pathogenic.

If one considers the large number of bacteria species which inhabit the skin and digestive tract of man, it is not surprising that some of the types found there could be capable of causing disease when the defense mechanisms of the host are weakened, or when the parasite somehow gains access to areas of the body in which it normally does not occur. For example, puerperal fever, a severe infection of the uterus which historically killed large numbers of postpartum mothers, results when normally harmless streptococci of the throat accidentally gain access to a damaged uterus, such as during childbirth under unsanitary conditions.

Microbial Flora of the Human Body

The characteristic flora of the human skin includes *Staphylococci*, *Streptococci*, *Cornybacteria* such as *C. diphtheriae*, and coliform bacteria. Yeasts and other fungi live in the moist areas of the skin. This flora is governed by nutritional conditions and by antibacterial properties of the skin, but no amount of bathing or scrubbing can totally remove these organisms. You are stuck with them for life.

The most common permanent residents in the mouth and throat are types of *Streptococci*, *Staphylococci*, *Neisseriae*, *Lactobacilli*, and the *Cornybacteria*. The same types of bacteria inhabit the nose.

These bacteria exist in a normally mutualistic situation in the human body, because by sheer numbers they can retard the establishment of virulent pathogens to which we are routinely exposed. However, under special circumstances these bacteria may take on pathogenic properties.

Staphylococci

Staphylococci are gram positive cocci that tend to grow in irregular, grape-like clusters. They live as part of the normal flora of the human skin and upper respiratory tract. The most common infection caused by *Staphylococci* are skin lesions called "boils" or "pimples." The cocci can become firmly entrenched in a hair follicle, and their exotoxins accumulate and destroy surrounding cells, leading to pus formation, sometimes follicle, and their exotoxins accumulate and destroy surrounding cells, leading to pus formation. Sometimes the cocci are introduced to the blood stream and are carried to other parts of the body, potentially causing rashes, meningitis, osteomyelitis (bone infection), and other diseases. It is very difficult to treat *Staphylococci* skin infections, both because of the nature of the walled off lesions and because of the high rate of mutation of the cocci.

Staphylococci may also cause food poisoning. They thrive in any suitable food which is kept warm for several hours, such as custards, cream filled pastries, milk products, and meats. They produce a powerful exotoxin which, upon consumption in sufficient quantity, produces nausea, cramps, diarrhea, and vomiting. The symptoms persist for several hours, and while they usually are not fatal, victims have felt as if the end were near.

Purpose

A potential pathogen becomes an active pathogen due to changed circumstances. There may be a change in the environment, such as entrenchment in a dirty and clogged hair follicle or transport from the throat to the uterus. There may be a change in the host, such as a lessening of bodily defenses against infection because of emotional or physical trauma. Or there may be a mutation in the parasite itself, such as a change from a nonhemolytic bacterium to a hemolytic (blood destroying) bacterium. In this laboratory you will attempt to isolate a hemolytic (and potentially pathogenic) *Staphylococcus* from your throat.

Pre-lab

Supplies needed:

Equipment

microscope
inoculating loop
forceps
bunsen burner or other flame source
slides
eye dropper

Materials

blood agar plate
sterile saline blank
TSY deeps
95% alcohol
2 tubes 0.5 ml. sterile saline blanks
mannitol salts plate containing a
  pH indicator
gram staining materials
four kinds of antibiotic discs:
  penicillin
  dihydrostreptomycin
  chloramphenicol, and
  tetracycline
sterile throat swab
cotton swab
sterile petri plates

Special Preparations

1) The blood agar plates and the mannitol salts plates may be ordered from several suppliers. Refrigerate them immediately upon receipt and store no longer than six months.

2) Regular cotton swabs are readily available in grocery stores and pharmacies. Longer handled throat swabs may be available in your local pharmacy; if not, ask any medical laboratory or doctor.

3) The antibiotic discs may be ordered from several suppliers. Refrigerate them immediately upon receipt.

4) Refer to the special preparations sections of laboratories thirteen and fourteen for directions on making blanks, deeps, TSY agar, and the gram staining materials.

Time Required

The isolation of *Staphylococcus* laboratory requires one full classroom hour, in addition to two partial hours, plus preparations, discussion, and review.

Procedure: Partial Hour 1

Students need the following: blood agar plate, mannitol salts plate, both types of cotton swabs, flame source, inoculating loop, and sterile saline blank.

During this laboratory session the student will transfer personal throat and nose bacteria to petri plates for incubation.

Steps

A. Moisten a sterile long handled swab in the sterile saline solution. Press the swab against the inside of the tube to remove excess saline.

B. **It is best to work in pairs during this step. It is difficult to swab your own throat.** Swab your partner's throat, and roll the swab over one-third of the blood agar plate, taking care that all sides of the swab are rubbed against the agar surface. With a sterile inoculating loop complete the streaking to insure isolated colonies. Refer to laboratory thirteen for directions on this streaking procedure.

C. Moisten the other cotton swab as in step B above, and thoroughly swab the inside of one of your nostrils. It may be more convenient again to have a partner for this procedure. Roll this swab over one-third of the mannitol salts plate, and complete the streaking with a sterile inoculating loop to obtain isolated colonies.

D. Label both plates, and incubate, inverted, at 37° C for 24 - 72 hours.

Procedure: Hour 2

Students need all equipment and materials listed. The TSY deeps need to have been melted by heating in a boiling water bath in preparation for this laboratory session.

During this session the student will observe colonial growth on the agar plates and will set up an experiment to determine the effectiveness of different antibiotics on a chosen bacterial colony.

Remember, you are handling potential pathogens. Be very careful not to further contaminate yourself or others. When the laboratory is complete, gather the plates together and dispose of them completely after re-sterilizing.

Steps

A. Pour a TSY plate.

B. Examine the blood agar plate for different kinds of colonies. This population, which consists of aerobes or facultative anaerobes, is only a portion of the species of bacteria normally present in the throat. The others are anaerobes.

1. Look for colonies which are surrounded by an area of complete clearing of the red color, or hemolysis. Some of the genera which cause complete hemolysis are *Streptococcus*, *Staphylococcus*, *Neisseria*, and *Escherichia*. Make gram stains of several colony types. Label and draw them in the space provided.

a) Colony shape and color ______________________

Bacterial shape, gram stain, and planes of division ______________________

____

____

Diagram:

b. Colony shape and color ______________________

Bacterial shape, gram stain, and planes of division ______________________

____

____

Diagram:

c) Colony shape and color ______________________

Bacterial shape, gram stain, and planes of division ______________________

____

____

Diagram:

2. Look for colonies which have a greenish halo of partial clearing, called alpha hemolysis. Some of the organisms which cause alpha hemolysis are species of *Streptococcus* and *Diplococcus*. Make gram stains of several colony types, label and draw them in the space provided.

a) Colony shape and color ______________________________

Bacterial shape, gram stain, and planes of division ______________________________

____

____

Diagram:

b. Colony shape and color ______________________________

Bacterial shape, gram stain, and planes of division ______________________________

____

____

Diagram:

c) Colony shape and color ______________________________

Bacterial shape, gram stain, and planes of division ______________________________

____

____

Diagram:

C. Observe the mannitol salts plate for the presence of colonies surrounded by a yellow area caused by acid production. One of the criteria for a potentially pathogenic *Staphylococcus* is that it produces acid from mannitol. Compare this acid producing colony with the colonies on the blood agar plate. If the colony is truly a potentially pathogenic *Staphylococcus*, the colony on the mannitol salts plate should have a colonial appearance similar to a colony on the blood agar plate surrounded by an area of complete hemolysis. Both colonies should be white. Pick such a white colony, if present, and homogenize it in the tube of sterile saline.

3. Make a gram stain of the suspicious looking colony.

Colony shape and color ____________________

Bacterial shape, gram stain, and planes of division ____________________

____

____

Diagram:

D. Dip an inoculating loop into the saline suspension and streak the TSY plate poured during step A. Streak in three different directions to insure complete seeding of the entire agar surface. This type of streaking is illustrated in figure one.

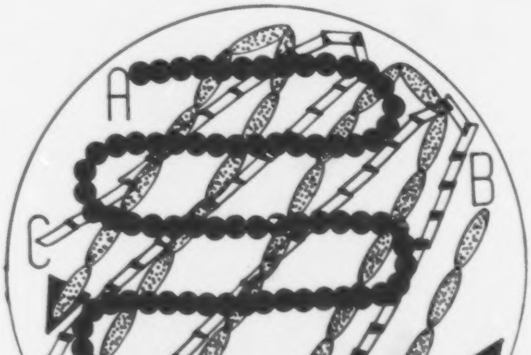

Figure 1

If the plate is thoroughly and evenly streaked in this manner, the resulting bacterial growth should completely cover the surface of the agar, without isolated individual colonies like those produced on the blood agar plate. The resulting generalized growth is called a bacterial "lawn."

4. What might be the advantages in research from such a growth pattern?

E. Dip the tips of a pair of clean forceps in alcohol and flame them. Pick up an antibiotic disc and drop it on the TSY agar plate about halfway between the center and the rim of the disc. Being careful not to touch the agar with the forceps, press the disc gently to insure good contact between the disc and the agar. Place the other three antibiotic discs in a similar manner, being certain that they are spaced apart from each other on the agar plate.

F. Incubate the plate, inverted, at 37° C. for 24 - 72 hours.

Procedure: Partial Hour 3

Students need the antibiotic plates incubated in hour two.

The bacterial growth should appear as a "lawn" covering the entire surface of the petri plate, with the exception of varying zones or areas of clearing around some of the antibiotic discs. These cleared areas are known as zones of inhibition, and their size is an indication of the effectiveness of the antibiotic. The larger the zone of inhibition, the more effective that particular antibiotic is against the variety of bacteria you have isolated from your respiratory system.

5. List the antibiotics in order of effectiveness, from most to least.

a) Most effective

b) Second most effective

c) Less effective

d) Least effective (or not at all effective)

6. Given your current respiratory bacterial flora, if you were ill with a sore throat, which antibiotic should a doctor prescribe for you? Why?

7. Compare your results with those of your classmates. Are their findings similar to your own? Why, or why not?

Resources

Abrahan, E. P. "The Beta-lactam Antibiotics," *Scientific American*, June 1981.

Adler, J. "The Sensing of Chemicals by Bacteria," *Scientific American*, April 1975 (#1337).

Braude, A. I. "Bacterial Endotoxins," *Scientific American*, March 1964.

Devoret, Raymond. "Bacterial Tests for Potential Carcinogens," *Scientific American*, August 1979 (#1433).

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Terminology

Students should understand the following terms and concepts prior to taking the unit review.

- host
- hemolysis
- lawn
- parasite
- pathogen
- *Staphylococcus*
- zone of inhibition

Review

15. Isolation of Staphylococcus

Name____________________________

Date_____________________________

Matching

______ 1. antibiotic
______ 2. hemolytic
______ 3. host
______ 4. gram positive cocci
______ 5. exotoxins
______ 6. trauma
______ 7. mannitol salts
______ 8. lawn
______ 9. anaerobes
______ 10. mutualistic

A. without air
B. acid production
C. pathogen
D. complete covering
E. normal body flora
F. chloramphenicol
G. man
H. *Staphylococcus*
I. lessened bodily defenses
J. food poisoning

Short answer

Why is it necessary to exercise great care in the disposal of incubated blood agar plates?

____

____

____

____

____

Answers Found: p 149 - #2, 3, 4, 5, & 10; p 150 - #1 & 6; p 151 - #9; p 154 - #7; p 155 - #8; Short answer: p 151.

16. Laboratory

Introduction to the Algae

When you have completed this laboratory you should be able to:

1. Identify algae phyla by physical characteristics.
2. Compare algae in terms of structure.
3. Diagram the life cycle of typical algae.

Purpose

Algae belong to the subkingdom *Thallophyta* which consists of plants lacking such specialized organs as roots, stems, and leaves. Algae species range from single celled plants to those with filaments, sheets of cells, or even large branched thalli. However, they share one common feature: with few exceptions, algae live in water, because they lack water conducting and nutrient conducting tissues. Nearly all plants which live in the ocean are algae, and many of the plants inhabiting fresh waters and ocean shores also are algae.

Although specialists differ on the taxonomical placement of algae, botanists generally consider that there are five primary phyla of algae grossly differentiated by color. You will have the opportunity in this laboratory to study representatives from these major algae groups. They are an important and often overlooked form of life.

Blue-green Algae

Blue-green algae belong to phylum *Cyanophyta*. These are the only prokaryotic algae, in that they lack an "organized" nucleus. Some place blue-green algae and bacteria with photosynthetic pigments in the same phylum, because their life cycle is similar in that they simply split in half to reproduce asexually. They may also reproduce by fragmentation of colonies or filaments, or by mitospores.

The *Cyanophyta* contain chlorophyll *a*, but not chlorophyll *b*, and they have as an accessory pigment phycocyanin, which gives them their characteristic color. Blue-green is the typical color, but because there are many variations between the species in the kinds and proportions of pigments, still other colors are possible. The Red Sea is said to have been so named because of the abundance of *Trichodesmium*, a blue-green algae which appears red. Because they are prokaryotes, the pigment is uniformly distributed throughout the cytoplasm of blue-green algae, and not in plastids. They commonly have gelatinous walls composed of pectic substances and store their food as glycogen, or glycoprotein, as animals do.

Some genera of blue-green algae are capable of nitrogen fixation, just like some bacteria. It has been established that they play a significant role in maintaining the fertility of rice paddies.

Certain bacteria and blue-green algae thrive at temperatures far higher than those which can be withstood by cells of other living things. Blue-green algae are common inhabitants of hot springs. In Yellowstone National Park thermal blue-green algae can live at temperatures of 90° C. (nearly the boiling point at that altitude), and they provide the brilliant colors seen in hot springs, geyser run-offs, and limestone terrace pools.

Blue-green algae are found in both fresh and salt water, although they are probably best known as the blue-green scum which can choke fresh water ponds and streams. Death and sickness to pets, livestock, wildlife, and even man have been attributed to blue-green algal toxins when concentrations in water supplies have become significant. The California State Water Resources Control Board, in their *Water Quality Criteria Handbook* (second edition), states that "there have been reports of rapid deaths of a great variety of animals after drinking water containing high concentrations of blue-green algae such as *Anacystis, Aphanizomenon, Nostoc rivulare, Nodularia, Gleotrichia, Gomphosphaeria,* and *Anabaena* — It is believed that such algae may be toxic to all warm blooded animals."

Green Algae

The green algae belong to the phylum *Chlorophyta*. These algae have pigments generally similar in kind and proportions to those of higher plants. They are eukaryotic and exhibit motility in diverse forms, from amoeboid movement to flagellation, and may be found as unicellular, colonial, or multicellular forms. They are mostly small, unicellular, globose, filamentous, or plate-like (sheets of cells). They may be septate, coenocytic (a continuous protoplast not divided into definite cells), or multinucleate. They reproduce both asexually by fragmentation or flagellated zoospores, and sexually by fusion of isogametes. In short, they exhibit all the variations possible in evolution from a unicellular organism.

They mostly inhabit fresh water, but they can occur many other places, such as in salt water and snowbanks (*Chlamydomonas* is the usual cause of red snow) and on turtles and sloths. Their food is stored as starch in specialized proteinaceous bodies called pyrenoids, like higher plants. Some produce oils instead of starch. The cell wall of *Chlorophyta* is in two layers; the outer layer is somewhat gelatinous and consists of pectic substances, while the inner layer is composed of cellulose, like higher plants. Their characteristic green color comes from pigments in the chloroplasts. They contain chlorophylls *a* and *b*, ∂ and β carotene, and the xanthophylls lutein and neoxanthin.

The *Chlorophyta* are a major source of food for aquatic animals (especially in fresh water). They play an important role in the oxidation ponds of sewage treatment plants, providing through photosynthesis the oxygen used by bacteria to decompose sewage. However, they, along with the blue-green algae, also can be a contaminant of fresh water supplies. The waterbloom which sometimes forms on the surface of reservoirs, lakes, and ponds in the summer is composed largely of planktonic green and blue-green algae.

Yellow-green and Golden-brown Algae

Yellow-green and golden-brown algae belong to the phylum *Chrysophyta*. They include the diatoms (which are diploid — all other *Chrysophyta* are haploid) and vary from unicellular to multicellular or colonial. They reproduce both asexually and sexually. Their characteristic color is yellow or brown, with accessory carotenoids of several kinds, including chlorophyll *e*, which is not known in any other plant group. They live in both fresh and salt water. Their food is stored as oils or as carbohydrates other than starch.

These yellowish algae, like other algae, are an initial link in the plant to fish food-chain, but otherwise the group generally has little obvious direct impact on man, excepting perhaps the diatoms. Diatoms not only make up most of the vegetable plankton of the cooler parts of the ocean, being therefore the most important base food source for fish and other marine animals, but since their "shells" are siliceous, these little creatures contribute even in death. Diatomaceous earth is an important resource, used in many things from toothpaste to blast furnaces.

Brown Algae

Brown algae belong to the phylum *Phaeophyta*. There are many large marine forms of *Phaeophyta*, commonly called kelp. Some brown algae are microscopic and filamentous, but most of them have a much larger, more complex thallus which can grow to more than 150 feet long. An accessory pigment called fucoxanthin gives them their characteristic color. The brown algae store their food as mannitol and laminarin, both soluble polysaccharides.

The brown algae reproduce asexually by fragmentation and by flagellated zoospores. They also reproduce sexually, exhibiting a distinctive alternation of generations from haploid (gametophyte) generation to diploid (sporophyte) generation, and back again. This alternation of generations is common to all the algal groups, with the exception of the *Cyanophyta*. Brown algae have heterogametes, that is, gametes in which the male and female are easily determined by size and other distinctions.

Algin, $(C_6H_80_6)_n$, is the common gelatinous outer pectic layer of the cell walls of brown algae, while cellulose is the main component of the inner layer. Algin is used in the processing of natural and synthetic rubber latex. Kelps are harvested as a major food source by the Japanese, and also as a source of the colloidal gel algin used in many prepared foods in Europe and America. Algin is used as a stabilizer or as a moisture retainer in a wide variety of commercial products, including ice cream, cake frosting, paints, and pharmaceuticals.

Red Algae

The red algae belong to the phylum *Rhodophyta*. They are quite variable in form and mostly marine and attached, rather than free floating. They are nearly all multicellular, forming complex, well branched thalli. Their cells are uninucleate to multinucleate.

Their characteristic color comes from an accessory pigment called phycoerythrin. Phycoerythrin absorbs especially well toward the blue end of the light spectrum, and since blue light penetrates the farthest through water, the red algae have an ecological advantage in being able to live more deeply submerged than their fellow algal groups. Their food reserves are usually stored as floridean starch (a substance which turns red or reddish violet when treated with iodine and which is unique to the *Rhodophyta*), soluble sugars, and sometimes fats. They are the most advanced and complex of all the algae groups.

Red algae are important sources of the colloids agar and carageenin. Prior to WWII, agar was produced almost entirely in Japan from the genus *Gelidium*. Since then several genera off the coasts of North America, Australia, and South Africa have been found to possess agar. Agar is widely used as a culture medium for bacteria (laboratories thirteen, fourteen, and fifteen), as a stabilizer or filler in many commercial foods (anyone prefer "homemade"?), and as a bulk producer in laxatives.

Carageenin is extracted from the red algae called Irish moss, *Chondrus crispus*. Carageenin is used as the emulsifying and stabilizing agent in many foods, such as chocolate milk and ice cream.

Pre-lab

Supplies needed:

Equipment

dissecting scope
microscope
teasing needle
forceps
watch glass
slides
coverslips
razor blade

Prepared Slides

Fucus - gametangia and gametes
Fucus - vegetative
Ulothrix - vegetative
Ulothrix - zoosporangia
Ulothrix - gametangium
Spirogyra - conjugation
Spirogyra - zoosporangia
Oedogonium - vegetative & zoospore
Vaucheria - vegetative & gametangia

Materials

tissues
algal samples
paper towels

Special Preparations

Samples of algae: if you do not live near the ocean, there are still many simple and inexpensive sources of algal samples. You will want to obtain at least five algal samples representative of the different groups.

1) Neighborhood ponds, streams, and other wet places are often a good source for blue-green and green algae. Collect samples as much as two weeks before the actual laboratory, and store in a cool but well lit area.

2) Fish tanks also have much to offer in the way of algal samples. It just depends on how rigorously the owners have scrubbed the tank walls.

3) Biological supply houses sell preserved specimens of all the major algal groups. One container will supply an entire class with a specimen.

4) Oriental grocery stores sell dried seaweed in many forms. One package of a type will supply classes for five years, so this is a very inexpensive source of samples. Large strips of kelp are available as "konbu" (Japanese); they are in the *Chrysophyta*. *Chlorophyta* and *Rhodophyta* are available as sheets of dried laver, or "nori" (Japanese). Hold a sheet up to the light to determine the color and hence the phylum.

To reconstitute dried algae, soak the laver or kelp in hot water for 20 - 30 minutes before running the laboratory. **Warning:** kelp reconstituted becomes five times the package size through absorption of water.

Time Required:

The introduction to the algae laboratory requires two classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the listed equipment, as well as the following materials: algal samples in numbered dishes and water.

You will be introduced to the wide structural variety of algae in this laboratory hour.

Steps

A. The teacher will have provided different species of algae in five numbered dishes. Prepare water mounts on slides with coverslips of small bits of algae taken from each dish and examine under the microscope.

1. Record the following characteristics for the unknown algae, #1 - #5, in figure one.

Characteristic	#1	#2	#3	#4	#5
Unicellular/ multicellular					
Filamentous/ plate-like/etc.					
Branched/ unbranched					
Cell shape					
Septate/ non-septate					
Nucleus/pro- or eukaryotic					
Color					
Plastids/present, number, shape					
Pyrenoids/present, number, shape					
Variation in form, cell type					
Wall markings					

Figure 1

2. Diagram a representative group of cells from each slide of algae. Identify and label the structures.

Algae #1

Algae #2

Algae #3

Algae #4

Algae #5

3. From your readings and observations this hour, determine the reasons for classifying these plants as *Thallophyta* and as algae.

4. Determine the phylum to which each of these algae belongs.

Algae #1

Algae #2

Algae #3

Algae #4

Algae #5

5. What characteristics are important in determining the phyla of algae?

It is interesting to compare algae of larger size with microscopic forms. The largest living algae are marine species. There are, however, many small marine forms; some of them are unicellular. The classification of algae is based on a complex series of characteristics involving form, reproduction, pigments, metabolic products, and wall constituents. In this laboratory you have been introduced to only the most obvious signposts of algal classification.

Procedure: Hour 2

Students need the listed equipment, as well as the following materials: tissues, the listed prepared slides, algal samples of *Ulothrix*, *Spirogyra*, *Oedogonium*, *Vaucheria*, and *Fucus*, if available.

Reproduction

Reproductive processes and structures are as variable as the forms of algae, and only a few samples can be considered during this hour.

The simplest methods of reproduction are asexual, such as the fission (dividing of an organism into two organisms) employed by simple algae. Other asexually reproducing algae multiply vegetatively by fragmentation (breaking into fragments which may develop into independent plants). Still others produce special reproductive cells called spores, each of which may produce a new plant. Spores with flagella or cilia are motile spores, and, since their motility is animal-like, the spores are called zoospores. Other kinds of spores also are named by special prefixes.

Typical Asexual Reproduction

The most common asexual spore is the zoospore, a motile flagellated cell which usually lacks a cell wall. The sporangium in which the zoospores are borne is usually derived from an ordinary vegetative cell; sometimes, however, it is a specialized cell morphologically unlike other cells. The entire protoplast of the sporangium may be transformed into a single zoospore. More often the protoplast undergoes one or more mitotic divisions, leading to the formation of two or up to a maximum of 32 spores within the original cell wall.

Zoospores are commonly released through a pore which develops in the wall of the sporangium. The zoospore swims about for a period from a few minutes to two or three days, depending on genus and species; one or two hours is the most common time. It then retracts or loses its flagella and secretes a wall, becoming a vegetative cell. In multicellular or colonial types, the vegetative cell then undergoes a series of mitotic divisions to produce a new organism or colony.

Zoospores are haploid or sometimes diploid. In addition to being borne in sporangia, they are sometimes formed by reduction division from a zygote, or by mitotic divisions from akinetes (an algal spore produced by the transformation of a whole vegetative cell).

Typical Sexual Reproduction

The sexual cycle of some algae involves alternating stages with N (haploid) and 2N (diploid) chromosomes. The 2N stage is brought about by the fusion of gametic nuclei, and the N condition is restored by reduction division. In many green algae, mitotic division occurs only during the haploid or N stage; therefore, the longest part of the algae's life will occur in this stage. Other green algae have mitotic divisions occurring in both N and 2N stages. A typical pattern might be as follows:

1) Gametes fuse to form a diploid zygote.
2) The diploid thallus develops.
3) Haploid cells (typically zoospores) are produced by meiosis. This affects some or all of the cells of the diploid thallus.
4) The haploid thallus develops from each zoospore by a series of mitotic divisions.
5) Gametes form within some or all of the cells of the haploid thallus.

This typical sequence of reproductive events is known as an alternation of generations. The N (haploid) stage is the gametophyte generation, and the 2N (diploid) stage is the sporophyte generation.

Ulothrix

In a green alga called *Ulothrix*, zoospores are produced in any cell of the filament. They are formed in groups of 4, 8, or 16. *Ulothrix* and *Spirogyra* are both examples of green algae, with mitotic divisions occurring only in the haploid stage.

Steps

A. Examine a prepared slide of *Ulothrix* and locate vegetative cells and zoosporangia (cells containing zoospores). Each zoospore has four flagella and, after being shed from the sporangium, swims for a while before becoming attached to the substratum and growing into a new filament. Details of spore structure are not visible in the slides.

B. Examine a prepared slide with *Ulothrix* gametangia. Often in the same filament housing zoosporangia, other cells may divide to produce 32 - 64 or 128 small motile-cells with two flagella. These biflagellate cells are called isogametes and the structure (cell wall) containing them is a gametangium.

6. In the space provided below, compare gametangia with zoosporangia.

7. Draw a vegetative cell, a zoosporangium, and a gametangium in the space provided below. Label your drawings.

Fusion of gametes results in a cell called a zygote. The zygote of *Ulothrix* develops a heavy wall and passes through a dormant period. The heavy walled resting zygote is called a zygospore. On germination, the zygospore undergoes meiosis; additional mitoses result in the formation of 16 meiospores. The meiospores are motile and are similar to the zoospores formed in the vegetative cells.

8. Why is the term meiospore preferred to zoospore at this stage?

9. Could the zoospores of the vegetative filament be designated as mitospores? Why or why not?

10. Label the diagram of the life cycle of *Ulothrix*, figure two.

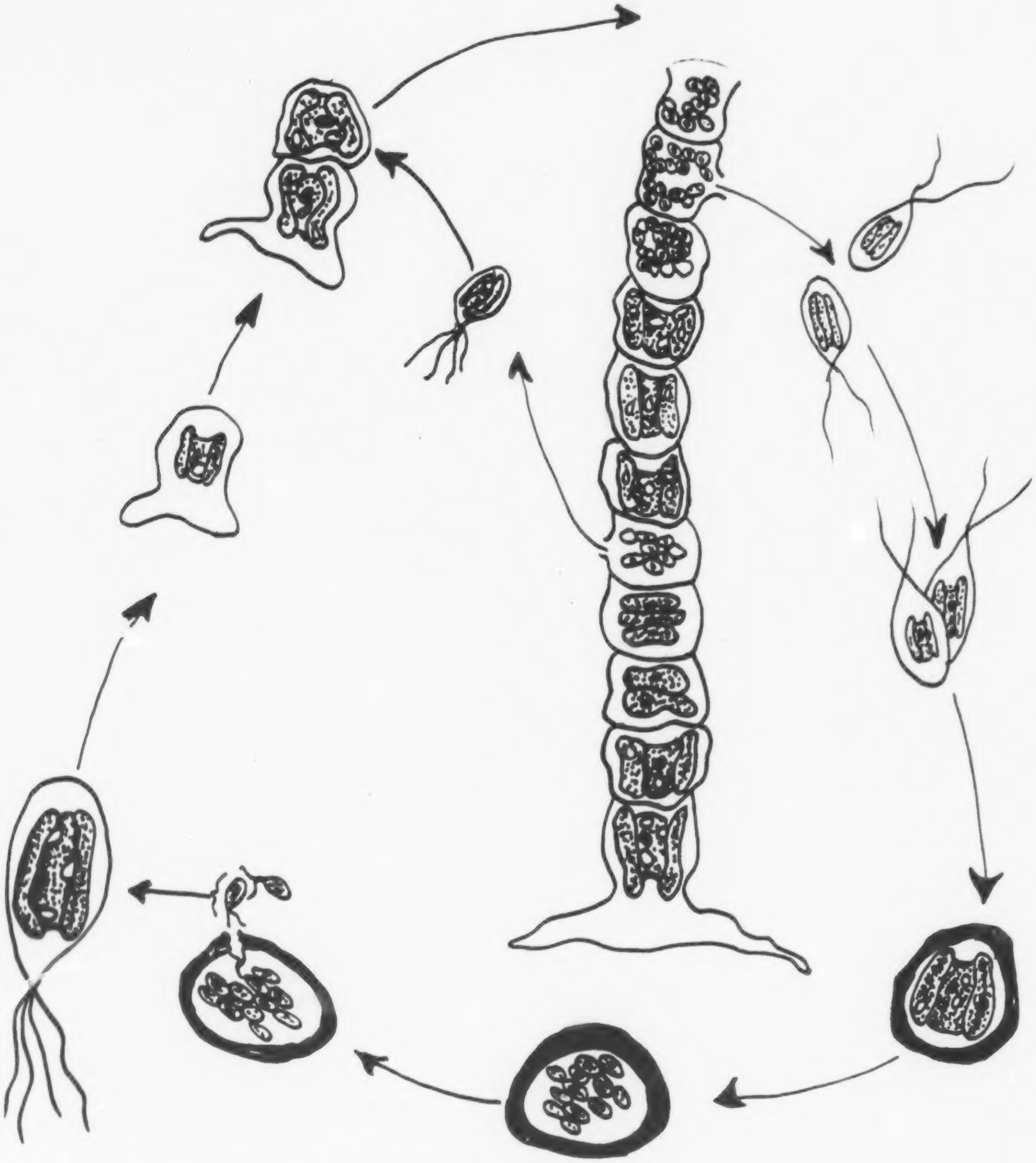


Figure 2

Fucus

C. *Fucus*, one of the *Phaeophyta*, is a rather complex alga in both form and life cycle. If specimens are available, examine the plant and note the branching thallus and swollen tips. These swollen tips contain gametangia of two kinds. In some species, the two sex organs are produced on separate plants; in others, they are formed together. Cut razor blade sections of the material and mount in water. Determine the kind(s) of sex organ(s) in your specimen.

11. Draw what you see in the space provided below and label the drawing.

D. Examine prepared slides of *Fucus* showing gametangia and gametes.

12. Are the gametes alike in size? ________

13. What term could be used to describe these gametes which would distinguish them from the isogametes of *Ulothrix*?____

____

____

The small motile male gametes fuse with large non-motile female gametes. The resulting zygote germinates and grows into a new plant.

14. Diagram a life history of *Fucus* with structures fully labeled. Vegetative, asexual reproduction may also occur from fragmentation of the thallus. Add the asexual cycle to the diagram of the sexual cycle.

15. Make a written comparison of the terms used in the reproductive cycles of *Ulothrix* and *Fucus* in the lines provided below. The terminology involves new terms which are essential to serious biology students. Learn these terms and the representative life cycles of *Fucus* and *Ulothrix*.

a. Asexual/sexual ______________________________

b. Mitosis/meiosis ______________________________

c. Diploid/haploid/monoploid/polyploid ______________________________

d. Gamete/isogamete/heterogamete ______________________________

e. Isogamous/heterogamous/oogamous ______________________________

f. Gametangium/antheridium/oogonium/sporangium ______________________________

g. Egg/sperm/zygote

h. Meiospore/mitospore/zoospore/zygospore

i. Conjugation/fertilization

j. Thallus/life cycle

Spirogyra, Oedogonium, and *Vaucheria*

E. Examine under the microscope slides of *Spirogyra, Oedogonium,* and *Vaucheria.* If specimens are available, also examine them.

16. In what characteristics of structure and reproduction do these plants differ among themselves and in comparison to *Ulothrix* and *Fucus?*

17. In what principal ways are these algae similar?

Additional Problems for Discussion

The following questions may be considered as post-laboratory activities. Your instructor may provide suggestions about avenues of inquiry, but you are to do your own thinking and organize your own work. You may want to consider outside resource materials in addition to your class notes and text.

18. Green flagellates (such as the *Euglena*, often studied in first year biology classes) are considered to be green algae by botanists. Asexual and sexual (both isogamous and heterogamous) production are known in the flagellates. Why would algologists place the flagellates in several plant phyla, although zoologists place them in one animal phylum — the *Protozoa?*

19. What evidence could be used to support the hypothesis that modern algae represent survivors and/or derivatives of geologically ancient algae groups? What objections to the hypothesis can you suggest?

20. What position do the algae hold in the food chain in a marine environment? What factors would you consider important in determining the distribution of algae in the oceans?

21. Why are characteristics such as wall material and stored foods considered to be so important in defining phyla of algae? Why is color only a superficial characteristic?

Resources

California State Water Resources Control Board. *Water Quality Criteria Handbook*. 2nd Ed., 1974.

Jackson, D. R., Ed. *Algae and Man*. Plenum Press: New York, 1964.

Smith, F. G. Walton, and Henry Chapin. *The Sun, the Sea, and Tomorrow*. Scribner: New York, 1954.

Terminology

Students should understand the following terms and concepts prior to taking the unit review, in addition to the terminology defined in question fifteen of this laboratory.

akinete
alternation of generations
Chlorophyta
Chrysophyta
coenocytic
Cyanophyta
filamentous
flagellate
fragmentation
gamete
Phaeophyta
plastid
plate-like
pyrenoid
Rhodophyta
septate
spore

Review

16. Introduction to the Algae

Name ____________________

Date ____________________

Multiple choice

______ 1. This phylum of algae stores food as a modified starch called floridean starch

a) *Phaeophyta*
b) *Chlorophyta*
c) *Rhodophyta*
d) *Cyanophyta*

______ 2. Sea kelp is found in this phylum of algae

a) *Chlorophyta*
b) *Phaeophyta*
c) *Chrysophyta*
d) *Rhodophyta*

______ 3. Diatoms

a) possess flagella
b) have siliceous walls
c) possess pseudopodia
d) belong to the *Rhodophyta*

______ 4. Larger forms of the red algae attach themselves to the substratum by means of a

a) pedicle
b) modified laminarin
c) flagellum
d) holdfast

______ 5. The compound laminarin provides

a) a method for food storage
b) for the production of a holdfast
c) increased photosynthetic efficiency
d) varieties of alginate

______ 6. Most algae found in fresh water, moist soil, rocks, and tree trunks belong to the group

a) *Chlorophyta*
b) *Cyanophyta*
c) *Chrysophyta*
d) *Rhodophyta*

______ 7. Alternation of generations implies that a particular species of plant does all of the below, **except**

a) has a meiotic stage which gives rise to gametes
b) produces haploid plants
c) restores its diploid number by mitosis
d) has a mitotic stage which gives rise to spores

______ 8. Some forms of kelp are processed so as to extract a component of their cell walls known as

a) carrageenin
b) laminarin
c) algin
d) floridean starch

Multiple choice

______ 9. Several genera of red algae have been found off the coasts of North America, Australia, and South Africa which provide additional sources for the production of

a) carrageenin
b) laminarin
c) alginate
d) agar

______ 10. The major source of food for aquatic animals in temperate ocean waters is

a) the *Phaeophyta*
b) diatoms
c) sea lettuce
d) *Protozoa*

Matching

______ 11. gametophyte

______ 12. asexual reproduction

______ 13. thallus

______ 14. plastid

______ 15. coenocytic

______ 16. pyrenoid

______ 17. filamentous

______ 18. septate

______ 19. akinete

______ 20. sporophyte

A. 2N

B. spore

C. not divided

D. divided

E. fragmentation

F. haploid

G. starch accumulates

H. photosynthesis

I. plant body

J. thread-like

Answers Found: p 159 - #13 & 14; p 160 - #3, 6, 15, 16, 17, & 18; p 161 - #1, 2, 4, 5, 8, & 9; p 166 - #7, 11, 12, 19, & 20.

17. Laboratory

Introduction to the Fungi

When you have completed this laboratory you should be able to:

1. List the classes of true fungi and their distinguishing characteristics.
2. Identify the most common fungi and their importance to man.
3. List the growth habits and reproductive characteristics of the fungal classes.
4. Compare the different classes of fungi with each other and with other plants.

Purpose

More than 40,000 species of fungi are known, and their large number is matched by their great importance to man. Together with bacteria, the fungi are the principal agents of decay in our world. Fungi are used for food, in medicine, and in industry. They cause diseases of man, other animals, and plants. It is as agents of plant diseases that fungi have their greatest economic impact, for fungi are as dangerous to plants as bacteria are to us.

Every student of the life sciences needs to have a working knowledge of the life habits, growth characteristics, and reproductive capabilities of the fungi. Sexual spores and life cycles, as well as asexual methods of reproduction, will be explored in this laboratory.

Fungus Classification

The phylum *Eumycophyta*, the fungi, is composed of four classes:

1) the *Phycomycetaceae* which reproduce sexually by öospores and zygospores;

2) the *Ascomycetaceae* which reproduce sexually by ascospores;

3) the *Basidiomycetaceae* which reproduce sexually by basidiospores; and,

4) the *Deuteromycetaceae* which represent the as yet unclassified fungi whose reproduction is known only in the asexual or imperfect stages.

Pre-lab

Much of the material for this laboratory can be obtained from nature. Students need to start saving material from the rotten world around them. Possibilities include moldy oranges, old bread, cheese, mushrooms, puffballs, and diseased plant leaves. It is amazing how many different kinds of fungi can be collected or grown in a three week period. Students, consider this a "sneak preview" of coming attractions.

Chytrid Traps

Students also need to devote one hour, either in the classroom or at home, to the construction and setting of a chytrid apple trap. Making the trap, as well as starting the collection of fungi, needs to be done approximately three weeks before the fungi laboratory. Students need the following materials for their traps:

- chicken wire
- wire cutters
- string
- apples

Make a wire "box" large enough to fit one apple. Cut the chicken wire approximately per the following diagram. Then simply fold until a cube has been formed.

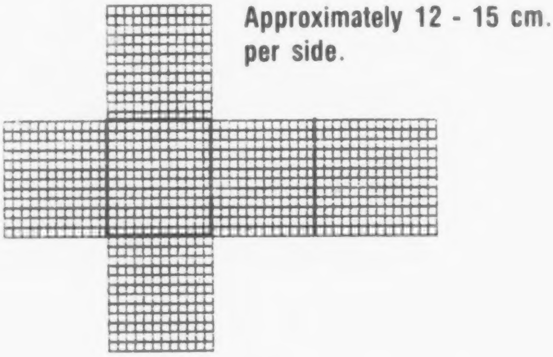


Figure 1

Take a bite out of the apple and put it into the wire cube. Chytrids are fungi that feed on fruit and live in freshwater ponds. The apple is bait, and the bite allows them ready access to the apple pulp. The chicken wire protects the bait from other apple loving creatures such as dogs, turtles, and fishes. Bend the wire securely around the apple, using the cut wire ends as fasteners, to form an approximate cube. Tie a strong string or rope of 10 to 15 meters in length to the apple trap. The trap is ready to be set.

Locate a body of either temporary or permanent water, preferably stagnant, and throw the chytrid apple trap into the water. Tie the end of the string to a solid object on the bank of the pond, and hide it from the hungry and curious of the neighborhood. Leave the trap for three weeks.

Once the students have collected home grown fungi and retrieved the chytrid apple trap, immediate preparations for the fungi laboratory can begin.

Other Supplies Needed

Equipment

- microscope
- teasing needle
- eye dropper
- slides
- coverslips
- forceps

Prepared Slides

- *Agaricus*
- *Claviceps* peritheca
- *Peziza* ascocarp
- *Puccinea graminis* spermagonia
- *Puccinea graminis* teliosorus
- *Puccinea graminis* uredosorus
- *Rhizopus* zygospores

Materials

live material collected by students
from their environment

chytrid apple traps
yeast

Note: If students live in a moist area, and it has not frosted, they may be able to locate a slime mold (*Myxomycophyta*). These creatures are not true fungi (*Eumycophyta*), but they are extremely interesting to observe. If a slime mold is brought to the classroom, put it into a moist terrarium with rotten wood and leaf mold, and watch its activities. Slime molds have been known to travel the length of a terrarium in one hour.

Time Required

The introduction to the fungi laboratory requires four classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the listed equipment, as well as the following materials: chytrid apple traps.

The Chytrids

These organisms are representative of the *Phycomycetaceae*, commonly called water molds. Water molds reproduce sexually by öospores in which the sexual spores are enclosed in a öosporangium, or little bubble like container. If environmental conditions are right, these spore cases may be collected. They look like this

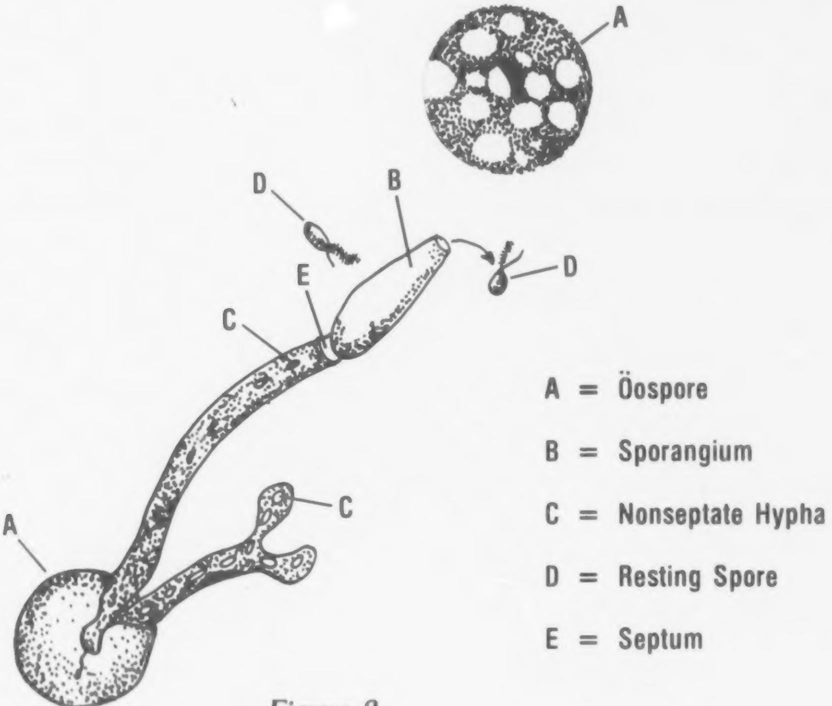


Figure 2

Some water molds can be very destructive, causing such diseases as potato blight and fish ick.

Steps

A. Take a teasing needle and obtain a small amount of pulp from the apple that was retrieved from the chytrid apple trap. Put the pulp on a microscope slide along with a drop of pond water. Cover it with a coverslip, and look at it under the low powered objective of the microscope.

B. Students will find many interesting forms of life. Attempt to identify all that are seen. Ask the instructor for help if necessary. Take care to categorize the life forms as to type, i.e. fungal, algal, protozoan, or insect. Draw identifications in the following chart, figure three.

1.

Fungal	**Algal**	**Protozoan**	**Insect**

Figure 3

C. Eventually students should focus the microscope on small, clear, round, single celled creatures living directly on the apple pulp. These are chytrids. Ask the instructor to verify the identification. Here is their taxonomical placement:

Subkingdom: *Thallophyta*
Phylum: *Eumycophyta*
Class: *Phycomycetaceae*
Order: *Chytridiales*

2. Chytrids have never been grown successfully in the laboratory. From their appearance and growth environment, what can you surmise about them?

3. Describe the life cycle and structure of chytrids and compare them with both algae and other fungi.

4. The phycomycetes as a group are known as algal fungi. Explain this terminology from your observations of the *Chytridiales*.

5. How might one devise a growth medium for chytrids that would allow them to live in the laboratory?

If you are interested in experimenting with the culturing of chytrids, devise a plan of action and see the instructor for materials and equipment. This experiment could be the start of a major research paper. Your instructor will tell you what is expected in your report. Remember to keep accurate notes on this experiment, so that it could be duplicated if successful.

Procedure: Hour 2

Students need the listed equipment as well as the following slides and materials: *Rhizopus* zygospores slide and the live material collected by students (black mold on bread or cake most likely would be *Rhizopus*).

Rhizopus nigricans

The organism *Rhizopus nigricans* is commonly known as bread mold, a member of the group called black molds. It is one of the most common and widely distributed of all fungi. *Rhizopus* is often seen as a black, cottony growth on bread and other foods. Black molds reproduce sexually by zygospores born on zygosporangia. They look like this:

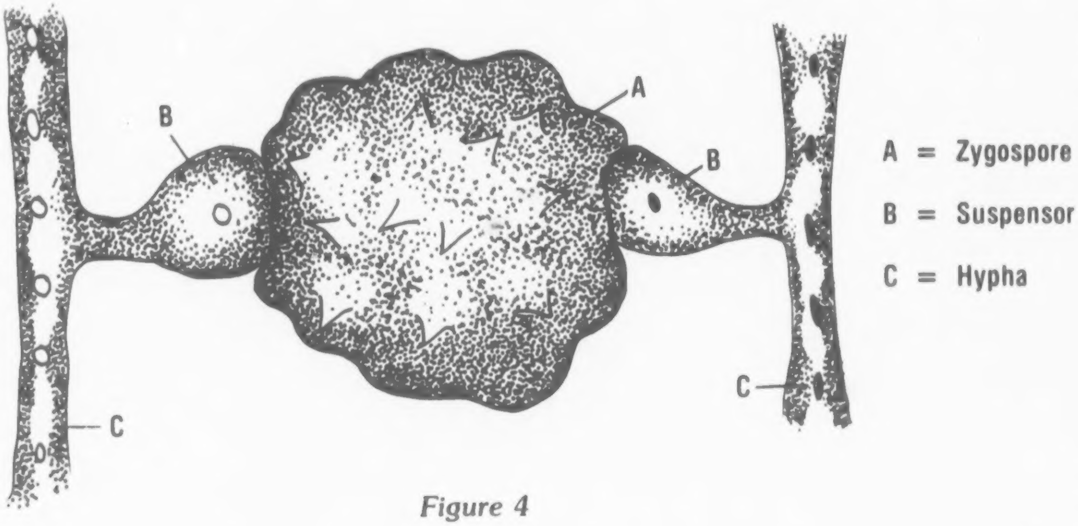


Figure 4

Some black molds can be very destructive and are a primary agent of putrifaction.

Steps

A. Place a piece of old bread infected with *Rhizopus* in water on a slide. Use needles to tease apart the bread. After the bread is finely separated, remove excess material and mount the preparation under a cover glass. Among the starch grains of the bread will be thread-like strands of the mold.

6. What color are these strands? ______________________________

7. Are they branched or unbranched? ______________________________

8. Septate or nonseptate (with cross walls or without)? ______________________________

9. Why may it be impossible to see the nuclei? ______________________________
____

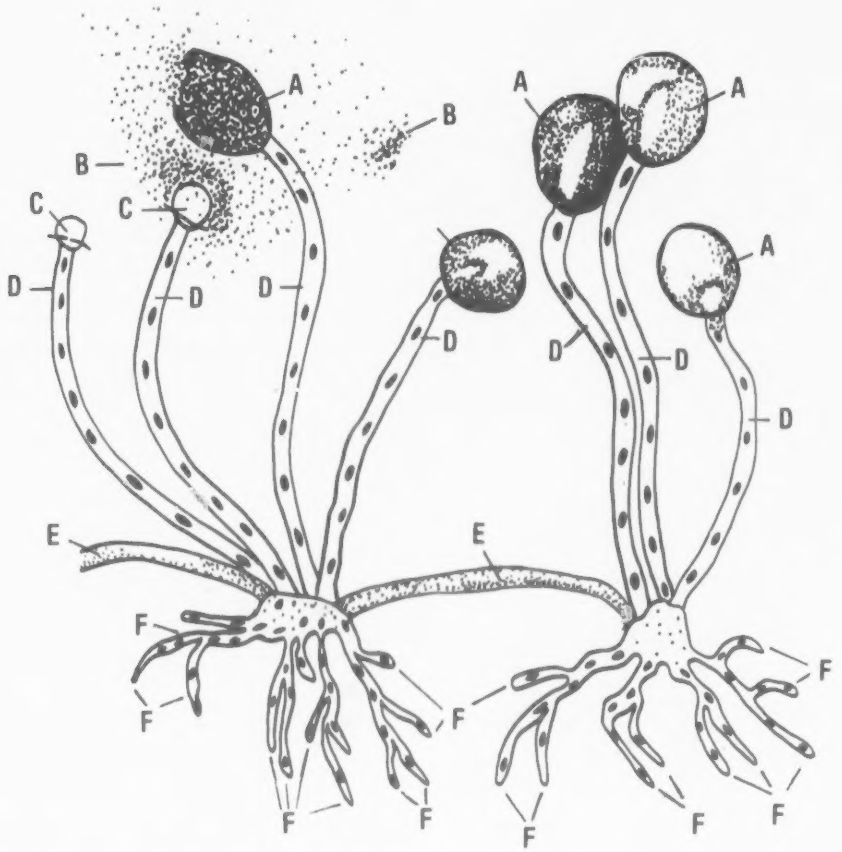
10. Are the contents uniform in appearance? ______________________________

11. Is there evidence of mold on the surface of the bread? ______________________________

12. What is the source of food for the mold? ______________________________

The thread-like strands of fungi are called hyphae (singular hypha), and the hyphae form a mass of strands called, collectively, the mycelium (plural mycelia).

B. Examine mycelia from several older bread cultures. Aerial hyphae may run parallel to the surface of the bread, or they may be erect. Erect hyphae usually grow reproductive structures that are asexual and are called sporangia (singular sporangium) at their tips. They look like this:



A = Sporangium
B = Spore
C = Columella
D = Sporangiophore
E = Stolon
F = Rhizoid

Figure 5

13. What steps occur in the development of the bulbous tip which ultimately becomes black as the spores mature?

14. Each spore may produce a new mycelium. These spores can also be called mitospores. Why?

Cultures of *Rhizopus* usually reproduce by means of mitospores. However, there are occasional wild cultures which produce sexually and form zygospores. In laboratories, molds are maintained by asexual subcultures. Strains of *Rhizopus* which are known to mate can be kept separately. Cultures can be prepared by placing spores or mycelia from known mating cultures at two sides of an agar substrate. The mycelia from the two strands appear structurally identical. Where hyphae of opposite strains meet, cell walls cut off gametangia, gametangia join, and the gametes fuse. The zygote enlarges and develops a heavy wall. This resting zygote is called the zygospore.

15. The zygospore, in germination, produces a hypha which is usually terminated by a single germ sporangium. Theoretically the germ sporangium should contain meiospores, which would produce both strains of the mold. Strains of this kind are described as + (plus) and − (minus) strains. Why is this terminology more desirable than male and female?

16. Since one cannot tell the difference in the + and − strains, is *Rhizopus* isogamous or heterogamous?

17. If spores were planted in the positions shown in the diagram below, what pattern would be formed by the zygospores? Draw the pattern on the diagram. The dotted lines indicate where the mycelia probably would meet.

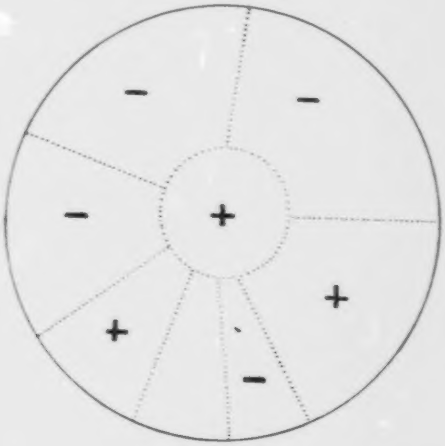


Figure 6

18. What method could be used for determining the strain of an unknown culture?

19. What reasons are there for suggesting the possibility that fungi are derived from algae?

20. Is *Rhizopus nigricans* a parasite or a saprophyte? ______________________

21. What may account for the rare occurrence of zygospores in wild cultures of *Rhizopus?*

____

____

____

____

Phytophthora Infestans

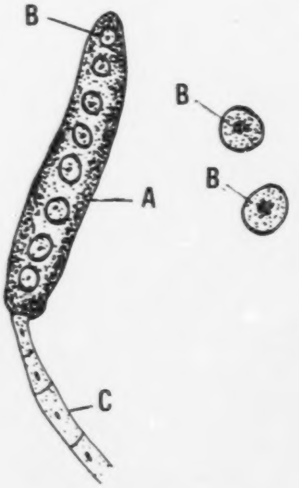
Another *Phycomycetaceae* organism causes late blight in potatoes. It singlehandedly changed the course of human history when it struck in epidemic proportions from 1845 - 1847 in Europe. It caused widespread famine and death, and, as a direct result, hundreds of thousands of Irish immigrated to the United States and South America. The genus and species name of this phycomycete is *Phytophthora infestans*. It also causes late blight of tomatoes. The symptoms are similar to late blight of potatoes, and the fruit is readily infected and subject to rot. Tomato blight occasionally appears in epidemic proportions in humid and hot areas of the United States. The phycomycetes, for very practical economic reasons, can not be ignored.

Procedure: Hour 3

Students need the listed equipment and the following slides and materials: *Claviceps* peritheca, *Peziza* ascocarp, yeast (if dried, dissolved in warm water with sugar or honey added), and student material (green molds on baked goods, cheeses, and oranges are often *Penicillium* mold).

The Ascomycetes

These organisms, also known as the ascus, or cup fungi, constitute one of the largest groups of fungi. They include the cup fungi, yeasts, powdery mildews, blue and green molds, and many others. They cause such important plant diseases as chestnut blight, Dutch elm disease, brown rot of stone fruits, peace leaf curl, apple scab, and vegetable soft rot. Cup fungi reproduce sexually by ascospores which are produced inside a saclike, enlarged end of a hypha which is known as an ascus. The ascus almost always holds eight spores and looks like this:



A = Zygospore

B = Suspensor

C = Hypha

Figure 7

Because of the antibiotic (against life) properties of some molds, a number of the ascomycetes are important bacteria fighting agents.

Steps

Penicillium

A. Examine cultures of *Penicillium*. It is often found as green mold on oranges and other foodstuffs. Mount some of the mycelium in a drop of water on a slide and cover with a coverslip. Examine under the microscope.

22. Compare its general growth habits with *Rhizopus*.

23. What is the difference in structure of the hyphae of phycomycetes and ascomycetes?

24. How are mitospores born on *Penicillium*?

25. Why are spores produced in this way given a special name, the conidiospores?

Students by now may have surmised that the fungus they have been watching is the source of the wonder drug penicillin. They additionally ought to know that *Penicillium* colors and flavors Camembert, Roquefort, and other cheeses.

Saccharomyces (Yeast)

B. Examine cells of yeast which have been grown in a nutrient solution composed of cane sugar and minerals. Each cell is a plant of yeast. In producing new cells, a chain of individuals may be formed.

26. In the material supplied, what proportion of the cells is separate?

27. Why does the grouping of yeast cells indicate "budding" rather than fission reproduction?

28. Under some circumstances, yeast may produce four or eight meiospores. The cell then functions as an ascus. Yeast cells which produce spores are diploid; the ascospores are haploid. Where does meiosis occur?

29. Vegetative reproduction (budding) may occur in either the diploid or haploid phase. What does this condition suggest in regard to the nature of spores and gametes?

Claviceps purpurea (Ergot of Rye)

C. Examine slides of ergot fruiting bodies and other specimens, if available. Compare with normal grains of rye. Notice that when the sclerotia (hardened knobs of mold) produce erect stalks, sexual reproduction is about to [illegible] place. The knob-like tips ("fruiting bodies," or stromata) contain peritheca, and the peritheca contain asc[illegible]

30. Contrast conidiospores and ascospores.

31. Why is *Claviceps* classified as an ascomycete?

32. In what sectors of agriculture might *Claviceps* be important?

It is fascinating to read reports of the important role *Claviceps* played in the witch trials in Salem, Massachusetts. Perhaps students would be interested in researching this historic intrusion of a fungus among us. Ask your instructor how to research and structure your report.

Peziza

D. Examine a "fruiting body," or ascocarp of *Peziza*, on prepared slides and, if possible, from nature. It represents only a small part of the plant. *Peziza*, or bird's nest fungus, grows in humus or decaying wood and produces visible cuplike growths above ground. Many branching hyphae are formed in the substrate long before the cuplike structures are formed.

33. Why might this be expected?

34. What functions would the subterranean mycelia serve?

35. Draw a diagram of the *Peziza* ascocarp.

36. Examine slides of the ascocarp under high power. On what surfaces are the meiospores born?

37. Why is it sometimes not possible to determine the number of spores produced?

38. Why is the spore-bearing layer called the fertile layer, or hymenium?

39. Determine, either from the prepared slide or from specimens, how many spores are born in a sporangium?

40. What is the shape of the spore containing structure?

An asci filled with ascospores has just been described.

The nuclear changes in *Peziza* are not adequately known. Another ascomycete, *Pyronema*, has been studied extensively and can be used as a model for a standard ascomycete life cycle. *Pyronema* is often discussed in standard textbooks.

41. From data supplied by lecture, text, or laboratory instructor, construct a labeled diagram in the space provided below to illustrate the sexual cycle of an ascomycete. (A typical life cycle diagram is shown on page 168.)

Procedure: Hour 4

Students need the listed equipment, as well as the following slides and materials: *Agaricus*, *Puccinea graminis* spermagonia, *Puccinea graminis* teliosorus, *Puccinea graminis* uredosorus, and live material (such as mushrooms, puffballs, lichens, and infected barberry or wheat, if available).

Basidiomycetes

The basidiomycetes are fungi that primarily reproduce sexually, and thus they are considered the most advanced class of true fungi, or *Eumycophyta*. They include the largest fungi and those that are best known. The meiospore of the *Basidiomycetaceae* is called a basidiospore and is borne on a basidium. It looks like this:

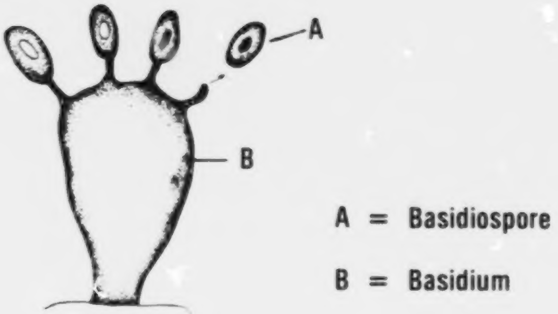


Figure 8

The *Basidiomycetaceae* are composed of two sub-classes which are very useful in categorizing the different types of basidiomycetes. They are:

Heterobasidiomycetaceae (differing basidia) which have the following orders—
- *Uredinales* (rusts)
- *Ustilaginales* (smuts), and
- *Tremellales* (jelly fungi);

Holobasidiomycetaceae (single or entire basidia) which have the following additional sub-classes—
- *Hymenomycetaceae* (mushrooms and allied fungi), and
- *Gastromycetaceae* (puffballs and their allies).

Steps

Agaricus

A. Examine fruiting bodies of *Agaricus campestris*, a species of common field mushroom, both as a prepared slide and fresh from the grocery store. The structure consists of a stalk (the stipe), and a cap (the pileus).

42. Where is the vegetative mycelia attached? ______________________________

____

B. The pileus consists in its lower portion of many radiating, blade-like gills. The gills consist of many hyphae with fertile tips. Remove a gill, or portion of a gill, and examine it for spores.

43. What name would be used to describe the layer of spore-bearing hyphae? ______________

44. Diagram the fruiting body of a mushroom and label its parts.

C. Spores are borne in groups of four. Each spore is borne on a short stalk or sterigma. The hyphal tip bearing the four basidiospores is known as a basidium.

45. Compare *Agaricus* with *Pyronema* (or *Peziza)* in terms of form, structure, and life cycle. Write in the following space.

Puffballs

D. Other basidiomycetes include puffballs such as *Fomes*, *Lycoperdon*, or *Polyporus*. Examine available specimens.

46. Compare a puffball collected from the wild with a mushroom such as *Agaricus*.

Lichens

E. Examine available specimens of lichens under the microscope.

47. Why are lichens classified as lichens and the components of a lichen classified as fungi and algae?

Many basidiomycetes are considered gastronomic delights. Mushrooms and their allied fungi are prized foods, as well as feared sources of poison. It depends on the species. For example, *Amanita caesareum* was used during the Roman empire to kill political enemies. On the other hand, the French and Italians go to great expense to obtain truffles for their cooking, and the Japanese scour the woods for *shiitake*.

Many diseases of plants are caused by basidiomycetes. Large sub-classes of disease producing forms are known by the common names "rusts" and "smuts." The conspicuous asexual spore of these fungi is the teliospore. This spore germinates to form an elongated basidium, which produces the basidiospores. Some rust fungi require only one host, while others require two hosts belonging to different species.

48. From your knowledge of the growth habits of fungi, discuss the weather conditions which might favor an epidemic of these diseases.

____

____

____

49. What effects do rusts and smuts have on their host plants? ______________________________

____

____

____

50. Are they considered parasites or saprophytes? ______________________________

Puccinea graminis

The fungus *Puccinea graminis* produces a disease of grains known as black, red, or stem rust. There are many races of the rust which affect wheat with varying degrees of severity. Development of resistant strains of wheat is sometimes hampered by the gradual but steady development of new races of the rust. *Puccinea graminis* produces five kinds of spores. Two of these, the aeciospore and pycniospore, are produced on barberry. Two, the uredospore and teliospore, are produced on wheat or other grasses. The fifth, the basidiospore, grows from the basidium and is not produced on any host. The life cycle of the rust is complex, but it must be known because effective controls of rust must be based upon its life cycle.

The leaves of infected wheat are often marked with reddish spots. This color is produced by red spores formed under the epidermis by the fungus mycelium. The spores are called uredospores, and the pustule is called an uredosorus. Uredospores may directly reinfect wheat plants.

F. Examine slides of wheat leaf showing uredosori. This stage in the life cycle is commonly referred to as the "red rust" stage.

51. Where is the mycelium which produces the spores? ______________________________

Each basidiospore may infect *Berberis* (barberry), but not wheat. In barberry bushes the basidiospores form mycelia which puncture the upper epidermis to form pycnia (a specialized spermagonia). The spermagonia, and the mycelia producing them, are of two kinds. Each spermagonium consists of slender hyphal tips and many minute cells called pycniospores (a special form of spermatia). Fusion of mycelial cells from the two types of spermagonia, or fusion of a spermatium with a hypha from the other mycelium, results in a diploid cell. The nuclei do not fuse. A binucleate mycelium develops from the binucleate cell. The binucleate mycelium produces "cluster cups," or aecia, on the lower side of the *Berberis* leaf.

G. Examine leaves of barberry for evidence of spermagonia and aecia, or examine prepared slides of the same.

52. How many mycelia are involved in their formation? ______________________________

These aeciospores spread to and infect wheat plants. The barberry bush is a classic example of an innocuous appearing plant which serves as an intermediary for a serious disease of an important agricultural crop. Another example is the role of gooseberries and currants in the transmittance of white pine blister rust.

53. The cycle of wheat rust is outlined in figure nine. Illustrate the structures named, include evidence of their nuclear conditions, and tell whether the rust is on a grass, barberry, or the ground during each stage of the cycle. Show your work on figure nine.

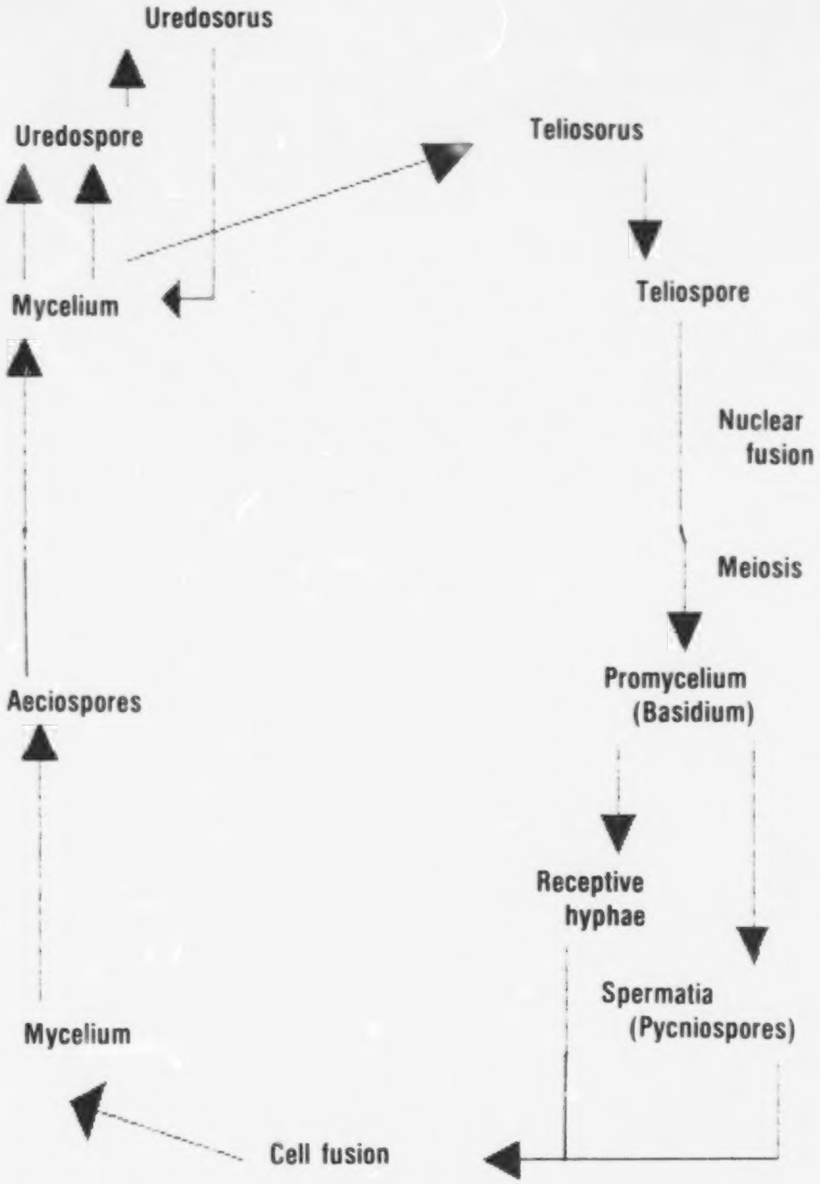

Uredosorus
Uredospore
Teliosorus
Mycelium
Teliospore
Nuclear fusion
Meiosis
Promycelium (Basidium)
Aeciospores
Receptive hyphae
Spermatia (Pycniospores)
Mycelium
Cell fusion

Figure 9

54. Do the nuclear phases coincide with the alternate host? ________________________

55. Why is winter wheat commonly less rusted than spring wheat? ________________________

____

____

____

56. Why has eradication of *Berberis* failed to control the disease? ______________________

57. Canadian winters are too severe to permit survival of the uredospores. In certain Canadian wheat growing areas, there also are no *Berberis* plants. Despite this, why are these areas sometimes visited with heavy infestations of wheat rust?

Students have now seen that *Puccinia graminis* produces five kinds of spores during its complex life history. No other fungus so threatens one of the world's basic food crops. As much as 25% of the world's wheat crop has been lost in a single year because of this parasite. The world's agricultural authorities have invested heavily in such rust control effort as improved agricultural practices, the wholesale destruction of barberry plants, and genetically breeding rust resistant strains of wheat.

Resources

Scientific American, September 1981. The entire edition of eight articles is devoted to fungi.

Strobel, Gary A. and Gerald N. Lanier. "Dutch Elm Disease," *Scientific American*, August 1981.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

aecia
Agaricus campestris
antibiotic
Ascomycetaceae
ascus
Basidiomycetaceae
basidium
Berberis
budding
Chytridiales
Claviceps purpurea
columella
conidium
Eumycophyta (true fungi)
fungus
Gastromycetaceae
gill
hymenium
hypha
lichen
mycelium
öospore
Penicillium
Phycomycetaceae
Phytophthora infestans
pileus
plus (+) and minus (−) strains
promycelium
Puccinea graminis
pycnia
Rhizopus nigricans
Saccharomyces
sclerotium
spermatium
sporangium
sterigma
stipe
stromata
suspensor
teliosorus
uredosorus
zygospore

Review

17. Introduction to the Fungi

Name ______________________________

Date ______________________________

Matching

______ 1. field mushroom

______ 2. black rust disease in rye or wheat

______ 3. puffballs

______ 4. water mold

______ 5. bread mold

______ 6. the wonder drug of WWII

______ 7. potato blight

______ 8. part algae, part fungi

______ 9. cup fungi

______ 10. yeast

A. *Phycomycetaceae*

B. *Penicillium*

C. *Ascomycetaceae*

D. lichen

E. *Agaricus campestris*

F. *Gastromycetaceae*

G. *Puccinea graminis*

H. *Saccharomyces*

I. *Phytophera infestans*

J. *Rhizopus nigricans*

Multiple choice

______ 11. When you eat a mushroom, you are eating the

a) reproductive structure of the *Basidiomycetaceae*
b) reproductive structure of the *Ascomycetaceae*
c) hyphae of the *Zygomycetaceae*
d) reproductive structure of the *Oomycetaceae*

______ 12. If a fungus obtains its food by feeding on dead organic material, then it is known as a

a) parasite
b) saprophyte
c) prokaryote
d) chemoautotroph

______ 13. A mass of hyphae is known collectively as

a) spores
b) branches
c) haustoria
d) mycelium

______ 14. Generally, the site of spore production in a fungus is called a

a) mycelium
b) fruiting body
c) hyphae
d) mold

Multiple choice

______ 15. The apple traps you made were for observing the fungus known as the

a) slime molds
b) bread molds
c) sac fungi
d) chytrids

______ 16. Each of the following is the site of meiosis and nuclear fusion in a fungal group except

a) ascus
b) basidium
c) stipe
d) teliosorus

______ 17. Yeast cells

a) bud
b) form conidia
c) longitudinally divide
d) form pseudopodia

______ 18. During sexual reproduction, a sac fungus forms a reproductive structure known as a

a) conjugation tube
b) conidium
c) basidium
d) ascus

______ 19. The most advanced and best known group of fungi are the

a) *Phycomycetaceae*
b) *Basidiomycetaceae*
c) *Ascomycetaceae*
d) *Deuteromycetaceae*

______ 20. The pileus is also known as a

a) cap
b) veil
c) stem
d) gill

Answers Found: p 178 - #15; p 179 - #4; p 181 - #5; p 182 - #13; p 185 - #7, 9, 12, & 18; p 186 #6, 10, & 17; p 190 - #1, 11, 14, 16, 19, & 20; p 191 - #3 & 8; p 192 - #2

18. Laboratory

Survey of the Mosses

When you have completed this laboratory you should be able to:

1. List the orders and classes of some *Bryophyta* and their distinguishing characteristics.
2. Identify the most common *Bryophyta* and their growth habits.
3. List the reproductive characteristics of the *Bryophyta*.

Purpose

Mosses are interesting not only because they are primitive land plants, but also because they have life cycles with a distinctive alternation of generations. This is a condition peculiar to some plants in which there is a sporophyte phase that produces spores, alternating with a gametophyte phase that produces gametes such as sperm and egg. Mosses are considered to be the most primitive of the higher plants. The mosses, belonging to the phylum *Bryophyta*, sub-kingdom *Embryophyta*, are plants with multicellular antheridia (male organs producing sperm) and archegonia (female organs producing eggs), but they lack specialized conducting tissues such as xylem and phloem like other higher plants. The *Bryophyta*, like algae and fungi, do not possess true roots, stems, or leaves.

The sporophyte generation attaches to the gametophyte and is partly or wholly parasitic on it. When we think of a moss plant we think of the haploid plant, because the diploid plant is simply an appendage on the larger, photosynthetic, and conspicuous gametophte.

There are three classes of mosses, the common mosses *(Musci)*, the liverworts *(Hepaticae)*, and the hornworts *(Anthocerotae)*. The hornworts are the least common, and in this laboratory you will study the structure and reproductive cycles of the two more common classes, the *Musci* and the *Hepaticae*.

Pre-lab

Supplies needed:

Equipment

dissecting scope
microscope
teasing needle
eye dropper
watch glass
slides
coverslips
razor blade

Prepared Slides

Mnium archegonia — longitudinal sections
Sphagum — archegonia
Marchantia — antheridial "branch"
Marchantia — archegonium
Sphagum — antheridia
Polytrichum — antheridium
Marchantia — capsule

Materials

tissues
fresh *Bryophyta* materials

Special Preparations

There are several ways to obtain fresh *Bryophyta* materials:

1) Fresh or preserved materials may be ordered from biological supply houses.

2) If you live where mosses and liverworts grow, have students collect as many types as possible in the fall of the year. Put these plants in a terrarium and water occasionally. The mosses will develop the sporophyte capsule during the winter, if the room is heated and thus simulates spring.

3) Buy or collect the spores of *Bryophyta*. Soak an unglazed brick in water, and place it in a shady, cool place, in a pan sufficiently deep to keep half the brick wet when filled with water. Sprinkle the spores over the brick's surface, and in a month or two you will have sufficient moss plants for the laboratory. This brick will be an excellent device to grow a steady supply of moss plants over the years. If you want the plants to develop reproductive capsules out of season, simply remove some from the brick and place them in a terrarium in the heated classroom four to six weeks before you wish to study the plants.

Time Required

The survey of the mosses laboratory requires two classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need all listed equipment and materials, as well as the following slides: *Mnium* and *Polytrichum*.

You will observe the structures and life cycle of common mosses during this laboratory hour. *Mnium* and *Polytrichum* are typical mosses which belong to the order *Bryales*, class *Musci*, phylum *Bryophyta*, subkingdom *Embryophyta*.

Steps

A. Examine available moss plants for general characteristics of the plant body. Carefully wash a small specimen, mount it in water on a watch glass, and examine it under a dissecting microscope.

1. What kind of structure anchors the plant and serves as an absorbing organelle? ____________________

____

2. What is its color? ____

3. How many cells compose it? ____

4. Why is it not called a root? ____

____

5. How are the "leaves" arranged on the axis? ____

____

6. How thick are the "leaves"? ____

7. What is the range in size of the moss plants available for study? ____________________

____

B. Dissect an antheridial cluster from the top of a *Polytrichum* or similar species. The flower-like tip of some plants contains the antheridia. By removing the center of the rosette, and separating the parts with a teasing needle, some antheridia will be observable.

8. How can the antheridia be distinguished from moss "leaves"? ______________________________

____

9. Are there any sterile structures among the antheridia? ______________________________

10. Are these antheridia structurally similar to antheridia of *Thallophyta* such as those studied in laboratory sixteen? ______________________________

11. Can you distinguish empty and full antheridia? ______________________________

C. Take and hold an antheridium so that the tip is between your thumb and forefinger. Simultaneously squeeze the tip and roll it between your thumb and forefinger. Submerge the tip of the leafy shoot in a drop of water on a slide and tease out the fragments from the tip of the shoot with a teasing needle. The male sex organs are simple, saclike structures. They produce large numbers of sperm cells. Place a coverslip on the slide, and observe the slide under first low power, and then high power of a microscope.

12. How many sperms (approximately) are produced in an antheridium of this moss? ______________

D. Compare these antheridia with sectioned antheridia in the prepared slide of *Polytrichum*.

13. Draw and label the parts of an antheridium of *Polytrichum* in the space provided below.

E. Examine prepared slides of longitudinal sections of a plant of *Mnium*. The secions are from plants which produce archegonia. At the top, in a rosette comparable to the antheridial cluster of *Polytrichum*, are egg containing structures mingled with sterile cells. Some of these structures will expose the egg. The jacket of cells enclosing the egg is extended into a long neck-like structure. The neck, if sectioned near the middle, will show a row of cells enclosed by the neck.

14. Why is it rarely possible to show, in section, a whole archegonium? ______________________________

____

____

15. What is the essential difference between an oogonium (studied in laboratory sixteen) and an archegonium?

____

____

16. Since the sperm are motile (able to swim), how would they reach the archegonium?

17. Where would they enter an archegonium?

18. What would happen to the cells contained in the neck, before the sperm reach the egg?

19. What connection does the zygote bear to the antheridial and archegional plants?

20. If the zygote were to undergo cell division indefinitely, what would be the source of food for the new diploid cells?

21. What would be the source of water?

22. How could the sources of food and water be determined experimentally?

F. Examine moss plants bearing capsules. These capsules are produced by the growth and cell division of the zygote. The capsule is borne on a stalk, the seta, and is attached to the leafy plant by a bulbous foot. These three parts, foot, seta, and capsule, represent the diploid phase of the moss plant.

In some mosses, th archegonium enlarges with the growing capsule for a period of time. When the seta elongates, the archegonial tissue tears; the lower portion remains as a basal cup, and the upper part forms a hood (calyptra) on the top of the capsule.

23. Why are the capsules associated with a leafy plant?

24. Would the cells of this archegonial hood (calyptra) be haploid or diploid? Why or why not?

G. Certain cells of the capsule undergo meiosis and produce meiospores. Examine slides of a capsule which you have made by sectioning a capsule and placing it in a drop of water with a coverslip. The darker streaks of cells are the areas of spore formation.

25. What percentage of the capsule is used in the production of spores? ______________________

26. Why is spore producing tissue said to be fertile tissue in contrast to the rest of the capsule which is sterile tissue?

H. The capsule may have an elaborate mechanism for the dispersal of spores. In most mosses a small lid is formed at the apex of the capsule and at maturity, the lid, or operculum, is removed. Many capsules are rimmed with a series of long "teeth" which help in the discharge of spores. Remove the operculum and cut off the rim of the capsule. Mount this rim on a slide and observe the rather elaborate structure.

27. List several reasons why many moss plants do not bear capsules ______________________

28. The spores germinate and form thread-like green plants called protonemata (singular: protonema). The protonemata produce buds which grow into moss plants. How would you distinguish protonemata from filamentous algae? ______________________

29. The spore bearing phase of the moss plant is generally called the sporophyte. The antheridial and archegonial plants are called gametophytes. How do these phases correspond to the haploid and diploid phases?

30. Are the diploid and haploid phases of algae and fungi comparable with the gametophyte and sporophyte of a moss? How or how not? ______________________

31. Some mosses produce antheridia and archegonia on the same plant. What term would describe this condition?

32. What term describes the condition in *Polytrichum* where some plants are antheridial and others are archegonial?

Procedure: Hour 2

Students need all the equipment and materials listed, as well as the following slides: *Sphagnum* — archegonia, *Sphagnum* — antheridia, *Marchantia* antheridial "branch", *Marchantia* archegonium, and *Marchantia* capsule.

During this hour the student will observe the structures and life cycles of two more typical *Bryophyta*, the peat mosses and liverworts. Peat mosses belong to the class *Musci*, with other common mosses, but they are in the order *Sphagnales*. Liverworts belong to the class *Hepaticae*.

Steps

A. Examine plants of *Sphagnum* and compare them with common mosses.

33. How can you distinguish between a "branch" and a "leaf"?

34. Where are "branches" and "leaves" most closely spaced?

35. How does this arrangement compare with the position of leaves and branches on higher plants?

B. Remove a "leaf" from one of the plants, place it in a drop of water with a coverslip, and examine it under the microscope.

36. How many kinds of cells are there in the "leaf"?

37. How are they arranged?

38. Can you suggest any relation between structure and water holding capacity? Explain.

Archegonia and antheridia are similar to those of other mosses. They are borne on lateral "branches" in the apical region of the plant. Capsules are relatively rare. The axis of the archegonial cluster elongates and forms a stalk; the sporophyte itself does not form a seta. The sporogenous cells of the capsule occupy a small area near the surface.

39. Why is such a stalk not equivalent to a seta?

C. Examine plants of available liverworts and compare them with mosses.

40. Why is it possible to distinguish liverworts from algae?

41. Where are rhizoids (root-like structures) produced in liverworts?

42. Why are the "leaves" of mosses and "leafy liverworts" not considered true leaves?

D. Small cup-like growths are borne on many species. These cup-like structures on liverwort gametophytes contain small buds of gemmae (vegetative reproductive structures formed by mitotic divisions) which may grow into new plants. Using a teasing needle, remove some gemmae from one of the gemmae cups and examine in a water mount under the microscope.

43. Why are these structures not called spores?

E. Antheridia and archegonia are borne in many positions and on various kinds of specialized structures. The fertile portions of *Marchantia* are rather complex and specialized. In fresh or preserved specimens, examine the structures bearing antheridia and archegonia. They are erect, peltate lobes of the thallus, in contrast to the flat, thallose vegetative lobes.

F. Also examine prepared slides of sections of both an antheridial and an archegonial "branch".

44. On which surface are antheridia produced?

45. Compare the antheridium of *Marchantia* with that of *Polytrichum* in the space provided below.

46. Where are the archegonia produced?

47. How many eggs are produced in each archegonium?

48. Compare the position and orientation of the archegonia of *Marchantia* with the archegonia of *Mnium* in the space provided below.

49. Is there any possible advantage in the difference in position and orientation? If there is, what is it?

G. Examine sections of the capsule of *Marchantia*.

50. What evident similarity is there between the capsule of a moss and a capsule of a liverwort?

51. Where would meiosis occur?

52. What are the products of meiosis?

53. How would new thalli be produced?

54. Is *Marchantia* homothallic?

55. In the space provided below, construct and label the structures in a generalized gametophyte and sporophyte plant for the phylum *Bryophyta*.

56. Can you generalize in this fashion for the algal and fungal groups? Why or why not? ______________

57. Prepare a chart, figure one, showing homologies of a green alga, a moss, and a liverwort. Descriptions and diagrams may be used. The structures named in the chart are to be in order of their development in the growth of the plants. Two major "guideposts" are already placed on the chart.

GREEN ALGA	MOSS	LIVERWORT
Conjugation	Fertilization	Fertilization
Meiosis	Meiosis	Meiosis

Figure 1

Resources

Conrad, H. S. *How to Know the Mosses and Liverworts*. Wm. C. Brown: Dubuque, Iowa, 1956.

Foster, A. S. and E. M. Gifford. *Comparative Morphology of Vascular Plants*, 2nd Ed. W. H. Freeman: San Francisco, 1974.

Terminology

Students shound understand the following terms and concepts prior to taking the unit review:

antheridium
archegonium
Bryophyta
calyptra
capsule
foot
gemmae
liverwort
moss
operculum
peat moss
protonema
rhizoid
seta
thallus

Review

18. Survey of the Mosses

Name ______________________________

Date ______________________________

Multiple choice

_____ 1. Mosses belong to the phylum

a) *Plantae* c) *Tracheophyta*
b) *Musci* d) *Bryophyta*

_____ 2. In the mosses the structures which anchor the plant body and absorb water and minerals are called

a) roots c) rootlets
b) rhizoids d) rhizomes

_____ 3. The leafy stem of a moss plant is the

a) sporophyte c) bryophyte
b) archegonia d) gametophyte

_____ 4. Sperm are formed in the sac-like, short stalked

a) protonema c) archegonium
b) seta d) antheridium

_____ 5. The union of the sperm and the egg results in a

a) zygote c) spore
b) gamete d) spore mother cell

_____ 6. The zygote is a member of what generation of plants?

a) gametophyte c) sporophyte
b) haploid d) pepsi

_____ 7. Which one of these mosses is a parasite?

a) protonema c) sporophyte generation
b) calyptra d) gametophyte generation

_____ 8. Mosses are adapted to live in a land environment in that

a) they have means of vegetative reproduction
b) they have an alternation of generations
c) they hold water near their bodies
d) they are no more than three inches tall

______ 9. Liverworts have reproductive structures called

a) protonema c) seta
b) gemmae d) calyptra

______ 10. The following represents the diploid phase of a moss

a) seta, foot, and calyptra c) seta, foot, and operculum
b) seta, foot, and capsule d) operculum, foot, and calyptra

Essay

1. List the distinguishing characteristics of the phylum *Bryophyta* and the traits that characerize the classes *Musci* and *Hepaticae*.

2. Explain the life cycle of a typical moss and relate this pattern to survival on land. ______

Answers Found: p 197 - #1 & 3; p 198 - #2; p 199 - #4; p 200 - #5, 6, 7, 8, & 10; p 203 - #9; Essay #1: pp 197 & 203; Essay #2: pp 204 & 205

19. Laboratory

Introduction to the Club Mosses and Horsetails

When you have completed this laboratory you should be able to:

1. List the distinguishing characteristics of the *Psilophyta*, *Lycopsida*, and *Sphenopsida*.
2. Distinguish between sporophyte and gametophyte generations of *Lycopodium*.
3. Compare the *Tracheophyta* to the *Bryophyta* in terms of their growth and reproductive characteristics.

Background

The *Tracheophyta* are popularly known as the vascular plants. Their primary distinguishing features are vascular bundles, or tissues such as xylem and phloem, with which they conduct water and nutrients. Because of this system of transport and support, vascular plants achieve heights and mass well beyond that seen in the *Thallophyta* or *Embryophyta*. The sub-kingdom *Tracheophyta* is undoubtedly the most familiar of all the plant groups, in that it includes such plants as ferns, pines, grasses, onions, apples, and orchids.

In this laboratory you will explore some of the simplest vascular plants, the club mosses as well as the horsetails and *Psilotum* (no common name). These are the most primitive vascular plants, containing features more advanced than algae, fungi, and mosses; none of them, however, produces seeds or retains true roots like the higher vascular plants.

Classification

Both as a review of previous laboratories and as an introduction to the *Tracheophyta*, let us consider a classification scheme in which the kingdom *Plantae* is divided into three sub-kingdoms. This system represents an effort to organize the myriad species of plants on the basis of similarities and differences in structure and life cycles. Such differentiations and classification schemes are not absolute. You should be aware that some organisms which share a few characteristics are sometimes placed in different phyla, divisions, or even sub-kingdoms, because of still other important characteristics. Even with these reservations, such classifications greatly assist efforts to understand and comprehensibly organize the plant kingdom.

Kingdom — Plantae

Sub-kingdom — Thallophyta
- **Division — Algae**
  - **Phylum** — *Cyanophyta*
  - **Phylum** — *Chlorophyta*
  - **Phylum** — *Chrysophyta*
  - **Phylum** — *Phaeophyta*
  - **Phylum** — *Rhodophyta*
- **Division — Fungi**
  - **Phylum** — *Myxomycophyta*
  - **Phylum** — *Eumycophyta*

Sub-kingdom — Embryophyta
- **Phylum** — *Bryophyta*

Sub-kingdom — Tracheophyta
- **Division — Pteridophyta**
  - **Phylum** — *Psilophyta*
  - **Phylum** — *Lycopsida*
  - **Phylum** — *Sphenopsida*
  - **Phylum** — *Filicophyta*
- **Division — Phanerophyta**
  - **Phylum** — *Cycadophyta*
  - **Phylum** — *Coniferophyta*
  - **Phylum** — *Anthophyta*

Psilophyta

The *Psilophyta* are the most primitive of the *Tracheophyta*. Although they contain vascular tissues, they do not have true roots and often lack leaves. They reproduce by spores rather than seeds. However, the gametophyte and sporophyte generations are physiologically independent of each other at maturity, unlike the *Bryophyta*. Most of the *Psilophyta* are only known by fossil remains from the Silurian and Devonian age, but three species still exist. *Tmesipteris tannensis* is confined to Australia and some of the Pacific Islands. *Psilotum complanatum* and *Psilotum nudum* are widespread in the tropical and subtropical regions of both hemispheres. In North America, *Psilotum nudum* extends as far north as South Carolina.

In the *Psilophyta*, as in all the *Tracheophyta*, the diploid generation is dominant. Also, as in all the *Pteridophyta*, the haploid generation is subterranean, or nearly so, and very small compared to the diploid. In the *Psilophyta* the gametophyte lack chlorophyll and must obtain nutrients from a mycorrhizal association with a phycomycetous fungus. The *Phycomycetaceae* are the only fungal group known to have existed in the Devonian period, when the *Psilophyta* were most abundant and when this association likely developed. Fertilization of sperm and egg follow a pattern similar to the *Bryophyta* studied in laboratory eighteen.

When the sporophyte of a *Psilophyta* becomes established as an independent plant, a layer of cells is formed separating it from the gametophyte. This is called an abscission layer, because the disintegration of its cells detaches the shoot from the gametophyte foot. The gametophyte may persist for some time after the sporophyte has become established, but sooner or later it dies and degenerates. Stem growth is from the tip (apical growth) rather than from inserted groups of cells (intercalary growth), as can be seen commonly in the *Thallophyta* and in the kingdom *Animalia*. This life cycle pattern is common to all the *Pteridophyta*, and can be diagrammed as seen in figure one.

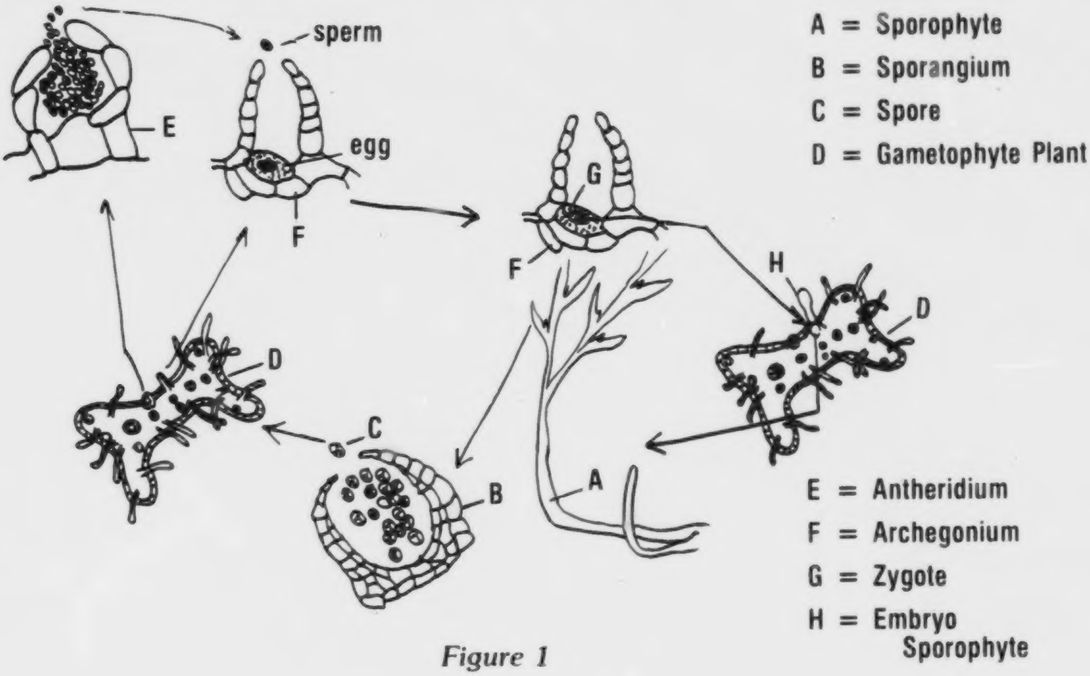


Figure 1

Lycopsida and *Sphenopsida*

The club mosses (*Lycopsida*) and the horsetails (*Sphenopsida*) are plants with a long history, extending from the paleozoic to the present, similar in this respect to the *Psilophyta*. The living members are of no great economic significance to man, but in the past many members of these two phyla were prominent. A knowledge of the structure and reproductive methods of the surviving members of these groups provides many clues to the extinct species, which can be studied only from their fossil remains.

The *Lycopsida* and *Sphenopsida* are spore-producing vascular plants with roots, stems, and leaves. However, in the *Lycopsida* the primary root does not persist to maturity, and therefore, upon digging, one finds adventitious roots arising from the internal tissues of the stem, or from stem-like organs called rhizophores. In both the *Sphenopsida* and the *Filicophyta*, a similar situation exists, but the underground horizontal stem is known as a rhizome, just as it is called in some higher plants like bluegrass and irises.

The roots of all *Pteridophyta* are not true roots at maturity. This is an important distinction from the *Phanerophyta*, along with spore production versus seed production, and still another reason to classify these plants together in the *Pteridophyta*. Their primary (known as initial) direction of growth is in one direction only, whereas in seed-producing plants, there are two initials of growth, those directions being the opposite of one another.

Lycopsida

The *Lycopsida* have only two living genera, *Lycopodium* and *Selaginella*. The *Lycopodium* are known as the club mosses, or ground pines. There are about one hundred living species, and they are all homosporous (producing one kind of spore). The *Selaginella* are known as small club mosses, or spike mosses. There are about five hundred species of *Selaginella*, and they are all heterosporous (producing two kinds of spores, one giving rise to male gametophytes and the other giving rise to female gametophytes).

The *Lycopsida* flourish from the tropics to temperate climates, especially in cool, moist, temperate forests. Many tropical species are epiphytic (grow above ground on other plants). The size of their branches varies from one to eight inches in height, and their horizontal stems elongate by apical growth, forming long runners. The stem in cross section reveals an epidermis, a wide cortex, and a pithless vascular cylinder composed of xylem and phloem. As the older parts die, younger branches continue to grow; thus, a single plant can give rise to several plants.

Lycopsid Structure

Lycopsida (club moss) leaves are small and numerous, and in some species so scale-like and small that the branches are mistaken for leaves. The place on the stem where a leaf is attached is called a node, and the part of the stem between one node and the next is called an internode. *Lycopsida* leaves are said to be alternate if only one is borne per node, opposite if two are borne, and whorled if three or more are borne per node. In most of the *Lycopsida*, the leaf has a small appendage on the upper side toward the stem. This appendage is called a ligule. Some botanists think that leaves may have originated from a modification of a dichotomously branched stem system, and that the ligule represents a vestigial branch in the primitive *Lycopsida*. Branching is always dichotomous.

Because the direction of growth is in one direction only, these plants cannot be transplanted unless the initial, or beginning point of sporophyte growth is transplanted as well. The author's mother attempted, several times, to transplant *Lycopodium* from a farm to her own home, only to continually fail, never realizing that the failure was due to a lack of true roots and the absence of the initial growth point in the transplanted sections of the plants.

Life Cycle

The spores of *Lycopsida* are produced in large kidney shaped sporangia borne singly in the axils of the sporophyll. In the more advanced club mosses the sporophylls are grouped into cone-like structures called strobili. Spores are formed from spore mother-cells after meiosis. They are tetrahedral with radiating lines on one side, and they escape the sporangium from a slit on its side, like the following:

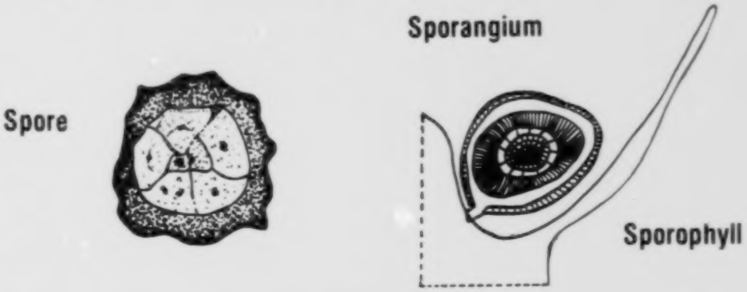

Sporangium
Spore
Sporophyll

Figure 2

The prothallus, or gametophyte generation of the *Lycopsida*, typically germinates below ground and lacks chlorophyll. The prothallus has a mycorrhizal association with fungi and is saprophytic on humus in the soil. It is thought that the associated fungus benefits from this association. The prothallus takes three to seven years to germinate, and another six to fifteen years to mature, depending on the environment and the species of *Lycopsida* involved.

The sex organs of the prothalli are borne on top, sunken in the tissues of the plant. The sperm are motile and need water to swim to the egg. After fertilization the diploid zygote produces the leafy plant. One prothallus may produce several plants and may persist for several years, ultimately being consumed by the sporophyte generation.

Modern *Selaginella* are heterosporous, and their life cycle follows the pattern shown in figure three.

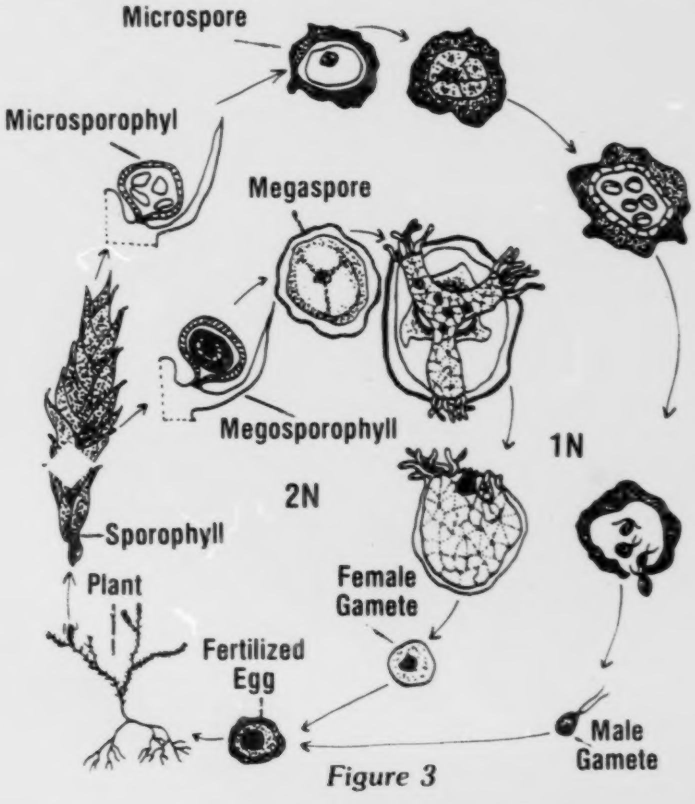

Microspore
Microsporophyl
Megaspore
Megosporophyll
1N
2N
Sporophyll
Plant
Female Gamete
Fertilized Egg
Male Gamete

Figure 3

Contemporary Importance

It is estimated that the *Lycopsida* composed up to a third of the ground cover of the pre-colonial forests of Eastern North America, and that lumbering and clearing for farms has massively reduced incidence of the phylum. The modern *Lycopsida* are of minor economic importance. Some of the *Lycopodium* are used in Christmas decorations, and both *Lycopodium* and *Selaginella* are occasionally grown for ornamental purposes. Some eastern states in the United States have placed restrictions on the harvesting of *Lycopsida* from public forests, because they are now endangered as a species due to heavy Christmas season harvests.

Selaginella lepidophylla is a species of the dry regions from Mexico to Peru, and it often is sold under the name of resurrection plant. In very dry weather the plant is dormant and forms a grayish brown ball, but when watered, it unrolls to form an attractive green plant.

Lycopodium spores, sold as lycopodium powder, are used in certain medications to form a bland cover and also to provide a natural bulk in laxatives (they absorb large volumes of water). They have also been used in the past as flash powder in the making of explosives.

Perhaps the major contribution of this group of plants derives from its extinct members. They were among the principal contributors to the vegetable deposits of the carboniferous period which were transformed into coal. Cannel coal, which burns with a bright and nearly smokeless flame, is derived largely from the spores of the *Lycopsida* and *Sphenopsida*.

Sphenopsida

The horsetails, or scouring rushes (*Equisetum*) are the only living representative of the *Sphenopsida*. Horsetail stems are hollow and jointed, and the leaves are borne in whorls at the nodes. When branches are present they also are borne in whorls. The leaves are so insignificant that most photosynthetic activity is carried on by the stems. There is no cambium in the stems, and vascular bundles are arranged in a ring. The stems contain a high concentration of silica.

Equisetum is homosporous, and the sporangia are borne on shield-like sporangiophores that are grouped in terminal strobili, like *Selaginella*. Spores fall on the ground and produce green photosynthetic prothalli with a number of erect green lobes. Both types of sex organ are produced on the same prothallus, and motile sperm bring about fertilization.

Equisetum is a popular plant in rock gardens, and thrives in moist areas.

Purpose

This laboratory is a descriptive exercise, similar to laboratories sixteen through eighteen, designed to introduce the student to the phyla *Psilophyta*, *Lycopsida*, and *Sphenopsida*.

Pre-lab

Supplies needed:

Equipment

dissecting scope
microscope
forceps
teasing needle
watch glass
slides
coverslips
probe

Prepared Slides

Lycopodia — gametophyte
Equisetum — gametophyte
Psilotum — gametophyte

Materials

Lycopodium
Equisetum
Psilotum
tissues

Special Preparations

1) There are several ways to collect a supply of *Lycopodium* for the laboratory, namely:

a) If you live in an area where *Lycopodium* grows, collect it in the woods;

b) If this laboratory is to be run during the Christmas season, many stores sell it as a decoration. Fresh *Lycopodium*, if stored in a cool, moist place, will keep for up to two months;

c) Or, if necessary, *Lycopodium* can be purchased from biological supply houses.

2) *Equisetum* (horsetail) is easily grown in a garden. Once installed, it does not require particular care and will be available for observation over the years. Horsetails, also known as scouring rushes, are available from local nurseries. They also grow wild in many areas.

3) *Psilotum* (no common name) is also easily grown potted. *Psilotum* is available from nurseries and also from biological supply houses.

Time Required

The introduction to the club mosses and horsetails laboratory requires one classroom hour, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need all listed supplies.

Steps

A. Study a *Lycopodium* plant with listed equipment.

1. What organs are distinguishable in the plant?

2. Where are the roots attached?

3. How large are the leaves?

4. How many veins are there in each leaf?

5. Describe the arrangement of leaves on the stems.

6. How do the leaves vary in size, color, and function?

B. Study the strobili and spores of the plant. The strobilus (reproductive cone) is a cluster of sporophylls (modified reproductive leaves which bear sporangia) which produce the spores. Their size and location will vary somewhat depending on the species under study.

7. Where, on the plant, are sporangia produced?

8. Where, in relation to leaves, are sporangia produced?

9. A leaf bearing sporangia is called a sporophyll. What term(s) would distinguish other leaves?

10. Sporangia produce many spores. What is the shape of a spore?

11. How are the spore walls ornamented?

C. Study the stem of your specimen.

12. What tissues are present in the stem?

13. What anatomical structure seems to sharply distinguish *Lycopodium* or club mosses from *Musci* or true mosses?

14. What structures seem to sharply distinguish club mosses from algae?

D. Observe gametophyte plants, either attached to your specimen plants, or with prepared slides, or both. The gametophyte is either a greyish, thick disc, or a wrinkled, lobed structure. The attached sporophyte consists of root, stem, and leaves.

15. What is the first haploid cell in mosses?

16. What is the first haploid cell in *Lycopodium?*

17. What would the first haploid cell in *Lycopodium* produce?

18. If the gametophyte were to grow very slowly, and to grow only after several years, why would it be found on the surface of the ground?

19. Since the gametophyte is not green, and is subterranean, what could be the source of materials for growth and respiration?

20. Since the sporophyte is attached to the gametophyte, where did fertilization occur?

21. What is the difference between a spore and a gamete?

22. Some species of *Lycopodium* have gametophytes which are aerial and green. Which type of gametophyte is more specialized? Why?

23. What are the differences between a sporophyte and a gametophyte of *Lycopodium?*

24. What are the differences between a leafy moss plant and the leafy young sporophyte of *Lycopodium?*

E. Compare *Equisetum* and *Psilotum* with *Lycopodium*, using observations similar to those required for the study of *Lycopodium*.

25. In three parallel columns, list characteristics which could be used to distinguish *Lycopodium* from *Equisetum* and *Psilotum*. Use the chart, figure four, upon which to list your observations.

Psilotum	**Lycopodium**	**Equisetum**

Figure 4

26. Do the differences you have listed in figure four support a strong difference in classification? ________

27. Where would **you** separate these three phyla? Why? ________

Resources

Margulis, Lynn. *Symbiosis in Cell Evolution: Life and its Environment on the Early Earth.* W. H. Freeman and Co.: San Francisco, 1981.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

Lycopsida
Phanerophtya
prothallus
Psilophyta
Pteridophyta
Sphenopsida
sporophyll
strobilus
Tracheophyta

Review

19. Introduction to the Club Mosses and Horsetails

Name______________________________

Date______________________________

Fill in the blanks

The spores of mosses and *Lycopodium* develop from

1. ____ enclosed in sterile jacket cells of the sporangium. The process by which they are formed is known as

2. ________________________. The eggs and sperm are cells formed within structures called

3. ________________________ and

4. ________________________. The process of gamete formation is called gametogenesis, in *Lycopodium* as well as in most plants. This process is called

5. ________________________ and results in the formation of

6. ________________________. Multicellular gametangia are borne on

7. ________________ which have developed from the germination of

8. ________________. The union of egg and sperm results in a cell called the

9. ________________, which grows to form the

10. ________________. The foot, seta, and capsule of a moss are comparable to the

11. ________________ of *Lycopodium*. The chromosome number of these structures is

12. ________________. In mosses, this structure is

13. ________________ upon the "leafy" plant, whereas in *Lycopodium* the structure is not, at maturity. In *Equisetum*, as in some of the more advanced *Lycopsida*, this structure is

14. ________________ and can persist for several years before being consumed by the

15. ________________.

Answers Found: In both laboratories eighteen and nineteen.

20. Laboratory

Survey of the Ferns

When you have completed this laboratory you should be able to:

1. List the distinguishing characteristics of the *Filicophyta*.
2. Trace the alternation of generations in ferns and compare it with other life cycles.
3. Compare homosporous and heterosporous ferns.

Background

Although many ferns are found in moist, shady areas, species of ferns grow in climates ranging from barren deserts to the frozen arctic. There are more than ten thousand species, and they range in size from eighty foot tall tree-ferns to microscopic plants. Ferns can be terrestrial, climbing, epiphytic, or even aquatic.

Humans use ferns variously. Ferns are used to decorate at holiday times and to landscape. A drug derived from a species of *Dryopteris* is a standard deworming medicine, especially used to expel tapeworm from the intestinal tract of man and other animals. In Asia, both *Athyrium esculentum* and *Ceratopteris thalictroides* are commonly used as a green vegetable, both raw and cooked.

Ferns can also be harmful. *Pteridium aquilinum*, bracken fern, is mildly poisonous to livestock. In some areas bracken fern forms a solid ground cover, taking over cleared, formerly productive grazing land. In Scotland alone there are more than 400,000 acres dominated by bracken fern. Recent studies also indicate that one of the reasons the Japanese suffer from an unusually high incidence of stomach cancer is that they regularly eat bracken fronds.

Classification

Ferns may be classified in seven distinct orders, four of which are extinct. Ferns all possess true roots, stems, and leaves, as do the more advanced *Phanerophyta*, but the ferns still reproduce by spores instead of seeds. The *Filicophyta* share the distinction with the rest of their pteridophyte relatives of being more numerous in the past than at present. The three modern orders are the *Ophioglossales*, the *Filicales*, and the *Marsileales*.

The *Ophioglossales* are commonly known as the adder's tongue ferns, and they are the most primitive of the modern ferns. There are about 90 species of *Ophioglossales*. They differ from all other ferns in that their sporangia are borne on a fertile spike which projects from the upper surface of the leaf near the junction of the blade and petiole. They are all eusporangiate. In eusporangiate ferns, the sporangium develops from a group of sporangial initial cells, just as in the more primitive club mosses. The jacket layer is more than one cell thick at maturity, and there is no highly specialized mechanism of dispersal.

The *Filicales* are known as the "true" ferns, with all members being homosporous and leptosporangiate. In leptosporangiate ferns, the sporangium develops from a single sporangial initial cell. The jacket layer is only one cell thick at maturity, and spore dispersal is usually due to hygroscopic changes in a group of thick-walled cells which commonly form a partial or complete ring called the annulus. As the sporangium dries out at maturity, tension develops within the annulus, which causes the sporangium to break along definite thin-walled cell lines. There are more than 9000 species of *Filicales*, and the common bracken fern, *Pteridium aquilinum* of the class *Polypodiaceae*, is the one that is often studied in biology classes.

The Marsileales group of ferns has no common name. They are heterosporous, leptosporangiate ferns which grow in water or wet places and are usually rooted to the substrate. People often do not realize that they are ferns. *Morsilea* look somewhat like a floating four leaf clover and are commonly called water clover. There are about 40 species of *Marsilea*, and, typical of the group as a whole, they produce two kinds of spores in sporocarps. Sporocarps are modified folded leaves or leaf segments which hold the sporangia of the plant.

One class of the *Marsileales*, the *Salviniaceae*, float on the surface of water instead of being attached. They include two genera, *Salvinia* and *Azolla*. *Azolla* is commonly called duckweed fern. Duckweed fern has engendered a lot of scientific interest in recent years because of its remarkable symbiotic relationship with the nitrogen fixing blue-green alga *Anabaena azollae*. They grow together at the surface of streams and ponds throughout tropical and temperate regions of the world. Like nitrogen fixing bacteria living inside the root nodules of legumes, the relationship is mutually beneficial.

Duckweed fern and its algal counterpart play an apparently important role in the fixation of nitrogen to the benefit of both themselves and rice growing man. For centuries, *Azolla* has been used as green manure in some areas of China and in other Asian countries to fertilize rice paddies. Some authorities believe intensified use of *Azolla* enabled the North Vietnamese to survive effects of the American blockade which disrupted imports of chemical fertilizer during the Vietnamese war.

According to Wilson Clark, writing in "China's Green Manure Revolution", the People's Republic of China has 3.2 million acres of paddy seeded with *Azolla*. This provides at least 100,000 tons of natural nitrogen fertilizer per year. Additionally, it is suggested that rice production increases as much as 158% with *Azolla*. Expanded use of *Azolla* could be an important factor in the world's future food supply.

Structure

The fern is a vascular plant with roots, stems, and leaves. The stems are perennial, living from year to year to produce new leaves each season. Excepting the tree-ferns, most fern stems are horizontal and grow underground. The root formed by the embryo does not persist and is soon replaced by many dark, wiry, adventitious roots which arise from the stem. Many ferns reproduce by vegetative means when creeping stems, called rhizomes, branch.

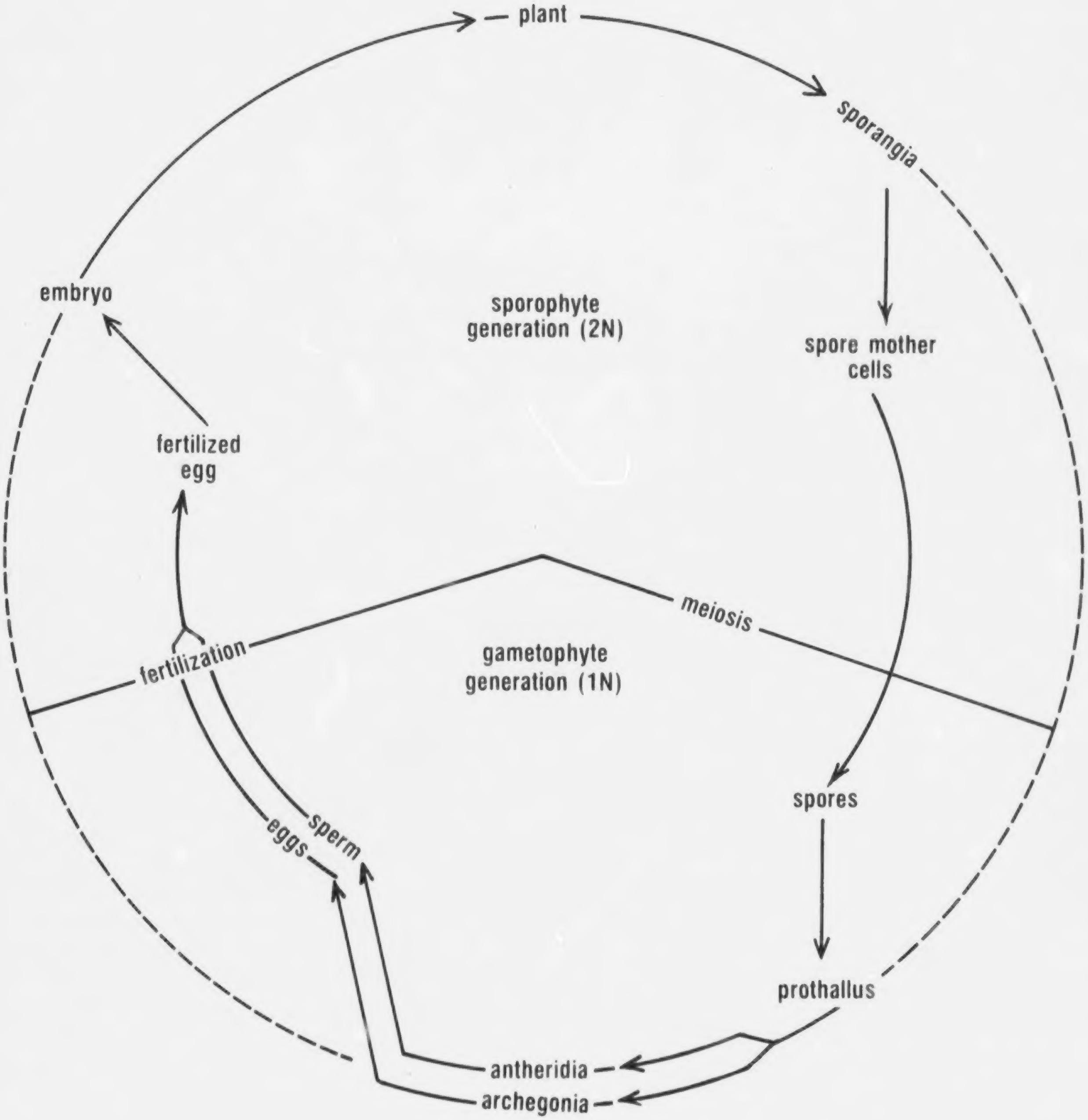
Fern leaves, called fronds, grow apically and begin in a coiled position which is called a fiddlehead. In a few species the extended fern leaf can be up to one hundred feet long!

Life Cycle

Fern spores are produced from special structures called sporangia, either eusporangia or leptosporangia. *Marsileales* spores are borne in sporocarps. Clusters of sporangia are called sori and may be naked or covered by an indusium.

The gametophyte of ferns, as with other pteridophytes, is called a prothallus. The prothallus varies in size and shape in different kinds of ferns, but it is always small and inconspicuous. In eusporangiate ferns it is often three to five centimeters long and many cells thick; but in leptosporangiate ferns the prothallus is usually thin, flat, and heart-shaped, seldom more than one centimeter long, and mostly only one cell layer thick. Rhizoids, antheridia, and archegonia are borne on the underside of the prothallus, with archegonia being closer to the middle of the heart's "notch" than antheridia. These structures are similar to those of other pteridophytes.

The life cycle of a fern follows the basic pattern shown in figure one.


plant
sporangia
spore mother cells
sporophyte generation (2N)
embryo
fertilized egg
meiosis
fertilization
gametophyte generation (1N)
spores
sperm
eggs
prothallus
antheridia
archegonia

Phases placed on the outer circle are multicellular, those on the inner circle are unicellular. The "meiosis" and "fertilization" lines separate generations.

Figure 1

Purpose

In this laboratory you will observe key characteristics of typical ferns and trace their reproductive cycles so that you can compare them with other pteridophytes. You also will compare homosporous and heterosporous ferns.

Pre-lab

Supplied needed:

Equipment

dissecting scope
microscope
watch glass
slides
terrarium
aquarium
probes
forceps
teasing needle
coverslips
eyedropper
razor

Prepared Slides

fern indusium
fern antheridia
fern archegonia

Materials

Marsilea with sporocarps
tissues
Azolla
homosporous fern gametophytes
several other fern specimens, some with sori

Special Preparations

1) Ferns are widely available from homes and gardens, local nurseries, garden clubs, universities, arboretae, and biological supply houses. Many will allow teachers to borrow the plants for a short period of time. Fern gametophytes are also available in the same manner. Maintain gametophytes in a moist environment, such as in a terrarium.

2) It may be necessary to purchase *Marsilea* with sporocarps and *Azolla* from biological supply houses, as they are not usually grown for decorative purposes. They are easily cultured and maintained in the classroom in aquaria.

Time Required

The survey of the ferns laboratory requires one full classroom hour, in addition to a partial hour, plus preparations, discussion, and review.

Procedure: Hour 1

Students need all listed supplies.

Steps

A. The sporophyte: examine available fern plants.

1. What organs compose the plant body?

2. Where does the axis increase in length?

3. Where is the oldest part of the axis?

4. Where are new leaves being produced?

5. What name should apply to the structure which includes the growing stem tip with its developing leaves?

6. Where are roots attached?

7. What name applies to an underground horizontal stem?

8. How do fern leaves contrast with leaves of *Lycopodium* and *Equisetum* studied in laboratory nineteen?

9. Do fern leaves grow in length? What evidence is there for this answer?

10. What terms would describe the leaf forms?

B. Slice a very thin cross section of fern stem, place it on a slide with water, and cover with a coverslip. Examine it with a microscope.

11. Diagram a cross section of a fern stem in the space provided below. Label the tissues. Is a cambium present?

C. Examine the under-side of fern leaves.

12. Are any of the leaflets different in appearance?

13. Diagram a leaflet showing veins and any other structures in the space provided below.

14. Compare the fern leaf with a branch of *Selaginella*, as remembered from laboratory nineteen. What possible homologies can you suggest?

D. Slice a very thin cross section of fern leaf, place it on a slide with water, and cover with a coverslip. Examine it with a microscope.

15. What tissues are present?

16. What kind of bundles form the vein system in this fern?

E. Observe the brownish areas present on some of the leaflets. Observe these areas with the aid of both the dissecting scope and microscope. Additionally study prepared slides of fern indusium.

17. Determine the structure of brownish areas present on some of the leaflets. By description and diagrams, illustrate the structures in the space provided below. Dissection and microscopic examination will help in making the necessary observations.

18. Compare the fern sporophyte with the sporophytes of *Lycopodium* and *Polytrichum*, studied in laboratories eighteen and nineteen.

E. The gametophyte: examine available gametophytes, as well as prepared slides of fern antheridia and archegonia.

19. Draw the under side of a gametophyte in the space provided below. Label apical notch, antheridia, archegonia, and rhizoids.

20. Complete the diagrams of sections of the antheridium and archegonium as drawn below. Label.

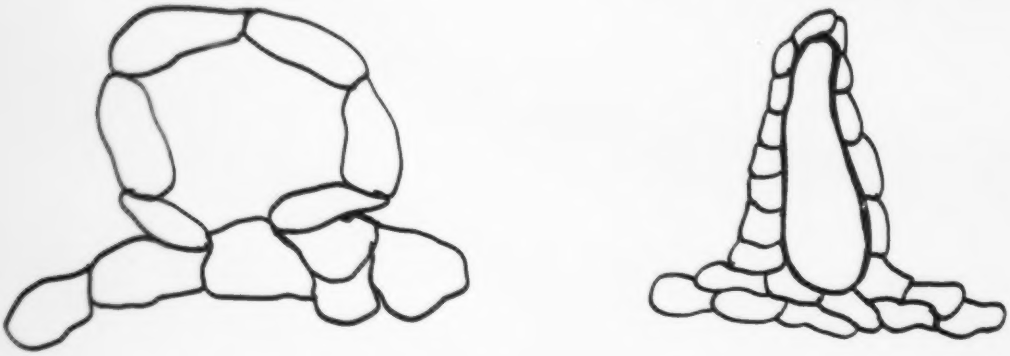


Figure 2

21. Explain the conditions which determine the attachment of the sporophyte to the gametophyte.

22. What conditions enable the sporophyte to become independent?

F. *Marsilea*: observe a specimen of this heterosporous fern.

23. Although *Marsilea* is unusual in appearance, there are many fern characteristics. What fern features are evident in your specimen?

G. Sporangia are produced in podlike leaf segments. These are called sporocarps. Prepare sporocarps for germination by gently squeezing them. Note the elaborate pattern of spore distribution. Scatter the spores in an aquarium for germination.

Procedure: Partial Hour 2

Students need all the listed equipment as well as the following materials: *Marsilea* and *Azolla*

Steps

A. Observe *Marsilea* gametophytes twelve to twenty-four hours old for sperms, archegonia, etc. Use both the dissecting scope and the microscope when making your observations.

24. Draw the two types of *Marsilea* gametophyte in the space provided below. Label all structures.

25. Contrast the sizes of the two kinds of gametophytes you have drawn in question twenty-four.

26. Contrast these *Marsilea* gametophytes with those of a homosporous fern.

27. Compare *Marsilea* with *Selaginella*, studied in laboratory nineteen.

B. Examine specimens of *Azolla*. Use both the dissecting scope and the microscope.

28. Diagram a specimen of *Azolla* in the space provided below. Label all structures.

C. Remove a dorsal leaf lobe and place it on a slide with a drop of water. Apply a coverslip with sufficient pressure to mash the leaf fragment. Observe under both low and high power objectives.

29. Under high power, the filaments of *Anabaena*, a blue-green alga that lives symbiotically in the leaves of *Azolla*, should be visible. You should also be able to see larger, oval cells called heterocysts within the filaments of *Anabaena*. Draw what you see in the space provided below. Label all structures.

30. How does the presence of *Anabaena* help *Azolla*?

In two to four days, your instructor may wish you to examine the young *Marsilea* sporophytes which will be in the aquarium.

Additional Problems for Discussion

The following questions may be considered as post-laboratory activities. Your instructor may provide suggestions about avenues of inquiry, but you are to do your own thinking and organize your own work. You may want to consider outside resource materials in addition to your class notes and text.

31. Young fern gametophytes, or gametophytes which are grown in crowded conditions, are likely to bear only antheridia. How may this be explained? What experimental work could be done to test your hypothesis?

32. Heterosporous ferns are similar to homosporous ferns. In constructing a diagram of a life cycle of a fern, what differences would be required by heterospory?

33. What excuse is there for using the word "leaf" for:

1) the lateral structures in moss gametophytes;
2) the simple leaves of *Lycopsida* and *Sphenopsida*; and
3) the complex leaves of the ferns?

Are they homologous? Defend your answer

34. If you wished to use radiation or chemicals to produce mutations in a fern, what part(s) of the fern could be used most conveniently? Why would controlled hybridization in homosporous ferns be difficult?

Resources

Clark, Wilson, "China's Green Manure Revolution," *Science 80*, 1(6), 1980.

Leonard, Johathan A., "Stalking the Mysterious Fern Seed," *Harvard Magazine*, May-June 1980.

Terminology

Students should understand the following terms and concepts prior to taking the unit review.

Azolla
eusporangiate
Filicales
fiddlehead
frond
heterosporous
homosporous
indusium
leptosporangiate
Marsileales
Ophioglossales
sorus
sporocarp

Review
20. Survey of the Ferns

Name ______________________________

Date ______________________________

Multiple choice

______ 1. Unlike bryophytes, tracheophytes have

a) a sporophyte generation dependent upon a gametophyte generation
b) no vascular tissue
c) leaves
d) no need for motile sperm

______ 2. An underground stem is referred to as a

a) rhizome
b) root
c) protonema
d) rhizoid

______ 3. The evolutionary advancement of possessing small roots and leaves is best represented by the

a) mosses
b) *Equisetum*
c) *Lycopsida*
d) *Filicophyta*

______ 4. *Filicophyta* possess

a) fronds
b) leaves
c) sporangia
d) all of the above

______ 5. The sporangia of ferns are located on what structure?

a) rhizome
b) frond
c) rhizoid
d) gametophyte

______ 6. Rhizoids are modified

a) roots
b) stems
c) leaves
d) flowers

______ 7. What land plant produces flagellated sperm and has as the dominant generation the diploid generation?

a) moss
b) fern
c) gymnosperm
d) horsetail

______ 8. The sporophylls of ferns produce reproductive cells

a) sexually
b) asexually
c) in alternate generations
d) seldom

______ 9. This fern enjoys a symbiotic relationship with *Anabaena*.

a) *Marsilea*
b) *Athyrium*
c) *Pteridium*
d) *Azolla*

______ 10. A certain plant is photosynthetic, stores starch as its principal carbohydrate, possesses multicellular reproductive organs with a lining of sterile jacket cells, undergoes its early embryonic development while still contained within the female reproductive organ of the parent plant, has no xylem, and produces no seeds. The plant is probably a

a) green alga
b) fern
c) conifer
d) moss

Matching

______ 11. covers sporangia

______ 12. group of sporangia

______ 13. an immature frond

______ 14. two spore sizes

______ 15. from groups of initial cells

A. fiddlehead

B. eusporangiate

C. indusium

D. sorus

E. heterosporous

Answers Found: p 221 - #1, 3, 7, & 15; p 222 - #2, 4, 6, 9, 11, 12, & 13; p 227 - #5; p 229 - #14; laboratory 19 - #10.

21. Laboratory

Introduction to the Gymnosperms

When you have completed this laboratory you should be able to:

1. List the distinguishing characteristics of the gymnosperms.
2. List the cell types of typical gymnosperm leaf, tissue, and bark.
3. Trace gymnosperm reproductive processes.
4. Compare gymnosperm growth and reproductive habits with ferns, club mosses, and mosses.

Background

In the plant division *Phanerophyta* there are three phyla, the *Cycadophyta*, the *Coniferophyta*, and the *Anthophyta*. The first two phyla are commonly known as the gymnosperms, or naked seed plants, and the third phylum as the angiosperms, or protected seed plants. These phyla include the most modern plants and those most commonly encountered. All reproduce with seeds.

The seed bearing plants came into being during the Permian period (225 to 280 million years ago) when there were worldwide changes of climate which involved stressful extremes. Terrestrial plants and animals were under strong selective pressures to evolve specialized structures which would make it possible for them to survive both freezing and drought. Just as amphibians gave way to scaly skinned lizards, so too did plants that reproduce with sperm and egg yield to seed plants. Some older forms remained, but seed plants became dominant.

In this laboratory you will investigate the two phyla of gymnosperms, the *Cycadophyta* and the *Coniferophyta*.

Pre-lab

Supplies needed:

Equipment

microscope
dissecting scope
hammer
probe

razor
small saw or serrated blade (now is the time for your Swiss Army knife)

slides
coverslips
watch glass
forceps

Prepared Slides

pine germinating microspores
Zamia microsporophyll
Zamia megasporophyll
Zamia leaflet
Ephedra stem cross section

Pinus leaf, cross section
Ginkgo staminate strobilus
pine ovules
Zamia stem cross section
Zamia petiole

Ephedra leaf cross section
Gingko stem cross section
Pinus stem, cross section
Gingko ovule

Materials

Zamia
Zamia seed
Thuja
Pinus edule seed
pine branch with leaves and clusters of small cones
mature pine cones
macerated pine wood
macerated pine bark
Pinus or *Juniperus* sections of wood
Gingko
Pinus
slab of a trunk, cross cut section
modelling clay
tissues

Special Preparations

1) Plant specimens may be obtained from individuals, nurseries, local universities, garden clubs, or biological supply houses. People usually are delighted to contribute plants for instructional purposes.

2) Pine materials may be obtained from the same sources or by taking a trip into the woods. Failing all else, you can chop your Christmas tree into pieces, wrap it in plastic, and refrigerate it for up to six months.

3) Wood sections should be cut in three planes, transversely, radially, and tangentially.

Time Required

The introduction to the gymnosperms laboratory requires four classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the following supplies: microscope, dissecting scope, slides, coverslips, watch glasses, probe, forceps, razor, tissues, *Gingko* and *Zamia* prepared slides, *Gingko*, *Zamia*, and *Zamia* seed.

The *Cycadophyta*

The cycads are gymnosperms with fern-like, usually large and pinnately compound leaves. The seeds are borne on modified megasporophylls (large, spore bearing leaves) which are usually arranged in a terminal cone. The stem has a large pith, a well developed cortex, and scanty vascular tissue. The stems are usually unbranched.

All modern cycads belong to one order, the *Cycadales*, and they are found only in tropical and sub-tropical regions of the world. There are three more, now extinct orders, in which one can trace the evolution of cycads from the ferns. One order, in fact, is known as the "seed ferns."

In this laboratory hour, you will investigate the cycads and the *Gingko*, the most primitive of modern gymnosperms.

Steps

The Sporophyte

A. Examine specimen plants of representative cycads, such as *Zamia*.

1. How might the plant parts be described? ________________________________

____

____

2. From observation of these plants, can you draw conclusions about their rate of growth? How?

3. In what ways are these plants fern-like?

4. What characteristics of the plant suggest structures suitable for arid climates?

B. Study the prepared slides of vegetative structures of cycads with a microscope.

5. What additional fern-like characteristics do you see with the microscope?

6. What characteristics are different from most modern ferns?

C. *Zamia* plants produce strobili at the apex of the stem. Either a staminate or an ovulate cone is borne at the stem tip. Study representative *Zamia* plants.

7. If the axis is to increase in length after cone production, where would this growth occur?

8. Compare ovulate and staminate strobili, sporophylls, and sporangia. Determine the position, arrangement, and number of sporangia.

D. In seed plants, the megasporangium usually is cloaked by one or two additional layers of tissue. These layers are called integuments. The megasporangium with integuments is called an ovule. The microsporophyll of *Zamia* and other seed plants is commonly called a stamen. Study slides of the reproductive structures of *Zamia*.

The Male Gametophyte

9. Microspores are produced in microsporangia and immediately germinate. Once multicellular, they are beyond the stage of being spores. Why?

10. What are these structures?

11. Study the prepared slide of sporangia containing germinated microspores. How many cells constitute the sporangia?

12. The male gametophyte is shed from the microsporangium before sperms are produced. At this stage it is called a pollen grain. What is the significance of the male gametophyte being enclosed within the spore wall?

13. How could the pollen grains reach ovulate cones?

14. What term is applied to the process of pollen transfer?

15. Why is this process considered very different from the process of fertilization?

16. Why are the two processes so generally confused?

The Female Gametophyte

17. In each ovule, only one cell produces meiospores. How many spores would be produced in each ovule?

18. Only one megaspore functions in producing a gametophyte. Since the spore is not shed from the sporangium, what relationship does the female gametophyte bear to the sporophyte?

E. Examine the ovules on *Zamia* plants containing mature female gametophytes.

19. How large are the gametophytes? ______

20. The archegonia are large enough to see without a microscope. They are small, pocket-like structures at one end of the starchy gametophyte. Why are they at the end nearest the place where the ovule is attached?

21. What is the fleshy coat of the ovule? ______

22. Is this fleshy coat completely closed? ______

23. An opening in the integument is called the micropyle. How close to the archegonia could pollen be brought by wind or insects?

Fertilization

The pollen grain is brought in contact with the megasporangium by evaporation or withdrawal of a "pollination drop" (a drop of liquid exuded through the micropyle). In contact with the megasporangium, the male gametophyte grows into the megasporangium. The cells of the megasporangium are digested partially by the pollen tubes, forming a pollen chamber in the apical region, and the male gametophyte gradually drops down close to the archegonia. The remnants of the tissue of the megasporangium collapse over the chamber. Two large multiciliate sperms are produced by each male gametophyte.

24. What liquid would be present for swimming sperms? ______

The union of sperm and egg results in the formation of a zygote. More than one zygote may be formed in an ovule.

Seed Formation

F. Examine seeds of *Zamia*.

25. Of what structures does the seed consist? ______

26. What are the interrelationships of sporophyte, megasporangium, megaspore, female gametophyte, and embryo sporophyte?

27. What functional specialization is accomplished by the formation of pollen and the process of pollination?

28. Compare cycad seeds with the seed-like development in *Selaginella*, studied in laboratory nineteen.

Gingko

G. Study both plants and prepared slides of *Gingko*, the maidenhair tree (named because its leaves resemble those of the maidenhair fern).

29. What comparisons can be made between *Gingko* and *Zamia*?

Procedure: Hour 2

Students need the following supplies: prepared pine slides, pine branches, trunk slab section, microscope, slides, coverslips, dissecting scope, razor, saw, and tissues.

Coniferophyta

Coniferous forests are best known as a source of lumber, ground cover, and occasional poetic inspiration. Although there are less than four hundred species in the phylum *Coniferophyta*, they constitute a significant percentage of the world's biomass. Their only serious competitors in the temperate regions of the world are deciduous angiosperms. And conifers dominate in sub-arctic regions. In the remaining three hours of this laboratory, consider how the form and function of the conifers contribute to their great success.

The conifers are quite variable. The following need to be considered during your studies of them.

1) Exactly what is meant by the term evergreen?

2) What is a particular species' general form, type of branching, size, and life span?

3) What type of leaves does it have — shape, size, arrangement, persistence, etc.?

4) Does the species have "short shoots," and what are they like?

5) What is the position, persistence, structure, and bract-scale complex of its ovulate cones?

6) What are the number, position, and form of its seeds?

7) What type of branching system does it use to maximize photosynthetic possibilities?

Gametophyte structures in all conifers are similar, but times involved in reproduction are variable from species to species. For your laboratory investigation, you will use *Pinus* as an illustrative form, partly because it is familiar and easy to obtain, and partly because it is the "classical" form against which other genera are compared.

You will consider the vegetative details of the sporophyte. You are to visualize the cells, tissues, and organs, three dimensionally. You will be asked to show your instructor that you can visualize in three dimensions by making perspective drawings and clay models. You also will consider the structures, timing, and sequencing involved in sexual reproduction. You will be asked to make comparisons between gymnosperms.

In this laboratory hour you will investigate the anatomy of gymnosperms.

Steps

Stem Structure

A. The stem of a pine is made of several tissues composed of different cell types. Microscopically examine stem cross sections, both in the prepared slide and a slide that you have made.

30. List the charactersitics by which you can identify the different cell types seen in the stem cross section.

B. The cells not only are representative of tissue types but also are restricted to rather definite zones. Cut a stem transversely with a razor blade. Examine the cut surface.

31. What common terms are used to describe a stem's visible zones?

32. Compare the common terms for structures seen with the naked eye, as listed in question thirty-one, with a stem cross section studied microscopically, as in step A. Which of the major zones is most complex in structure?

The center of the stem is composed of cells nearly round in cross section. The cells are not widely variable and are thin-walled. The zone is called pith, and the tissue composing the pith is called parenchyma.

Next to the pith is a zone of angular empty celled tissue, the wood, or xylem.

33. Are the xylem cells empty?

34. Are they all thick-walled?

35. Are there any differences in cell shape in the xylem? ____________________

The rows of cells elongated along radii of the stem constitute wood rays or xylem rays. The heavy walled, empty, angular cells are tracheids.

36. Which type of cell is oriented vertically in the stem? ____________________

37. How can you determine that tracheids are rather long cells? ____________________

The large intercellular spaces, surrounded by thin-walled living cells, are the resin canals. The cells surrounding the canals are the secretory cells.

The bark, composed of all the tissues outside the xylem, is the most complex zone. It is composed of several tissues and in older stems can be divided into zones of live bark and dead bark.

Most of the xylem is in radial rows because it is derived from successive mitoses of a ring of cells called the cambium. The cambium zone can be fairly well delimited at the edge of xylem. Since xylem cells gradually mature from new cells produced by the cambium, there is not a sharp demarcation of the cambium.

External to the cambium is a layer of phloem.

38. Do rows of ray cells continue from xylem through the phloem? ____________________

39. Are there other phloem cells arranged in radial rows which correspond to the rows of xylem cells? __

40. Since cambium is in line with both xylem and phloem, how can this arrangement be explained?

In the phloem are slender, angular, nearly empty cells called sieve cells. There are also broader, shorter cells called phloem parenchyma cells, and the radial series of cells composing the phloem rays.

Outside the phloem are large, thin-walled cells similar to the cells of pith. This tissue is parenchyma, and the zone is the cortex.

41. Are there resin canals in the cortex? ____________________

The surface of young stems is covered by a single row of cells, the epidermis. In older stems, an outer layer, the periderm, is usually produced from the epidermis. The periderm is several cells thick and consists, in part, of cork tissue.

42. Does the specimen supplied have an epidermis? What is the evidence for an epidermis? (If necessary, check this with your instructor.)

C. Compare the stem section with a slab cut from a trunk.

43. What tissue areas increase more as the stem grows into a trunk?

44. What special markings distinguish live bark from dead bark?

45. What tissues are spatially moved by increase in diameter?

46. Diagram and label a pine stem section in figure one below. Show the location of zones and tissue types (the section is a wedge-shaped, cross-cut portion of the stem, running from core to bark).

Diagram of pine stem section.

Periderm

Cortex
  Parenchyma

Phloem
  Sieve cell
  Parenchyma
  Ray

Cambium

Xylem
  Spring Wood
  Summer wood
  Tracheid
  Ray
  Resin canal
  Secretory cell
  Annual ring

Pith
  Parenchyma

Figure 1

47. Draw, in the space provided below, a few cells from each tissue type to illustrate shape, wall thickness, and content. Name the tissue and label each cell type.

Procedure: Hour 3

Students need the following supplies: wood sections, wood blocks, pine branches with needles, saw, hammer, macerated pine wood, macerated pine bark, pine needle slide, slides, coverslips, microscope, modelling clay, and tissues.

Steps

Pine Wood

A. Examine sections of wood cut in three planes: transverse, radial, and tangential. Study the wood cells as seen in these three views.

48. Model each type of cell with modelling clay. These models are to show cells as three dimensional objects. These models will be given to your instructor at the conclusion of this laboratory hour.

49. What is the shape of a whole xylem ray? ______________________________

50. What are the markings on the cell walls? ______________________________

B. Compare sections with wood blocks.

51. What structural characteristics of the wood determine the grain? ______________________________

52. Diagram the tissue areas in the wood block supplied for this laboratory. Show all macroscopic, or visible, structural characteristics in three planes, and label the planes of section. Use figure two below.

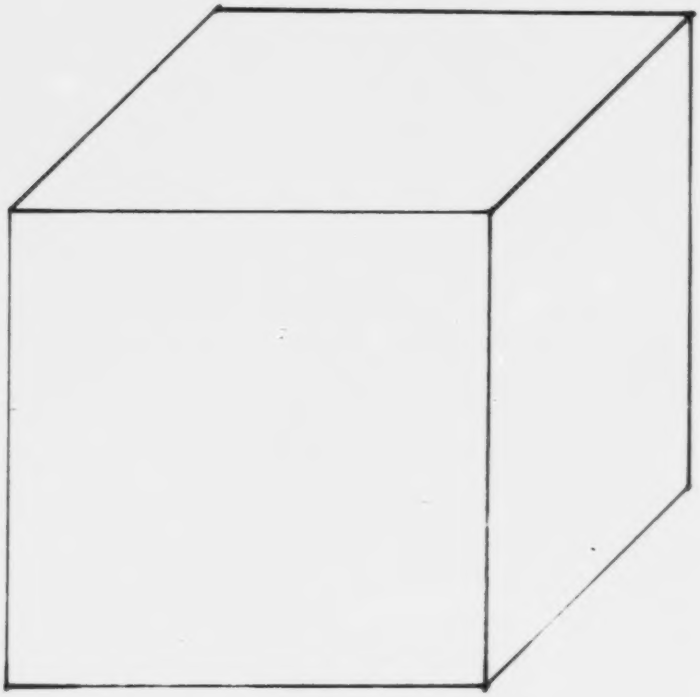


Figure 2

C. Examine macerated pine wood, both with the naked eye and with the microscope.

53. What cell types can you identify in this material? ____________________

54. What are the basis for identifying each cell type? ____________________

D. Examine macerated pine bark, both with the naked eye and with the microscope.

55. What bark cells can you identify? ____________________

56. If you were to examine bark materials, how would you determine if it were adulterated by wood? ____________________

E. Examine pine leaves, both live and with prepared slide cross sections under a microscope.

57. Sketch and label pine leaf cell types in the space provided below, using separate drawings for each cell type as you did for stem tissues. (You should have sketched the following cell types: epidermis with stomata, mesophyll parenchyma, endodermis, pericyclic parenchyma, xylem, and phloem.)

58. Diagram a cross section of a pine leaf in figure three below, showing areas of each tissue.

Figure 3

Procedure: Hour 4

Students need the following supplies: pine branch, pine cones, *Pinus edule* seed, microscope, slides, coverslips, tissues, razor, probe, and prepared slides of pine germinating microspores and pine ovules.

In this laboratory hour you will investigate the reproductive process in conifers.

Steps

Staminate Strobili

A. Examine a pine branch with leaves and clusters of modified leaves, or small cones.

59. What evidence is there that each cone represents a branch?

60. What are the structures composing each cone?

61. How many sporangia occur on each microsporophyll (stamen)?

62. How does the position of the sporangia compare with that in ferns?

63. How does the position of the sporangia compare with that in the *Lycopsida?*

B. Microscopically examine the contents of the sporangia by crushing a stamen in water.

64. Pollen grains are composed of how many cells?

65. Pine pollen grains are usually winged. The wings are not cells but represent air sacs within the cell wall. What evidence is there to support this statement?

66. Are the sporangia dorsal? What evidence can you cite for your answer?

67. How many cells are in mature pollen grains?

68. Why is the term pollen grain not just another term for a microspore?

69. What other term could be used for a germinated microspore?

70. Under what circumstances would pollen be shed from the sporangia?

Ovulate Strobili

D. Examine the pine branch again. Small cones are produced near the tips of branches. There may be one to four, depending on species, produced on any one branch. Also examine single, mature cones.

71. What difference in appearance is there between staminate and ovulate cones?

72. When pollen is shed from the microsporophylls, how can it be brought to the ovulate cones?

At pollination time, the megasporangium with a tubular jacket (integument) has not produced megaspores. The megasporangium with its integument is called an ovule.

74. If there is a space between the ovulate cone scales, how close to the ovules could pollen grains be carried?

Following pollination, the male gametophyte continues growth and penetrates the wall of the megasporangium. The megasporangium develops a single tetrad of spores, and only one megaspore germinates.

75. When the megaspore germinates, what structure would be produced?

Gametes are produced the spring following pollination. The sperm cells (microgametes) are produced in a tubular outgrowth from the pollen grain. The eggs (megagametes) are produced singly in archegonia at the edge of the female gametophyte. Fusion occurs after a sperm cell from the pollen tube is discharged into the archegonium.

76. What changes occur in the cone during the year between pollination and fertilization?

77. What is the relationship between the female gametophyte, the sporangium, and the plant bearing the sporangium?

78. When the zygote germinates, what will it produce?

79. What relationship does the new sporophyte have with the:

gametophyte?
sporangium?
integument?
parent sporophyte?

The Seed

Accompanying the growth of the young sporophyte, the strobilus continues to enlarge. At maturity, the ovule and its contents are called a seed.

80. Where are seeds borne in the mature pine cones?

81. How many seeds are produced on each cone scale?

E. Dissect a *Pinus edule* seed. The blunt end of the seed is the region of attachment to the cone scale. The somewhat pointed end is the micropylar end, or, the micropyle (the region where the integument is open and where pollen grains came in contact with the ovule).

82. Draw in the space provided below the dissected seed and show its observable parts. Indicate the probable position of the archegonium in relation to the formation of the embryo. Draw arrows showing where the terms to be found in the right hand column below are to be found in your seed diagram.

Seed Coat (hardened integument)

Remnants of Megasporangium

Female Gametophyte

Cotyledons of Embryo

Root Tip of Embryo

Stem Tip of Embryo

Micropyle

Position of Archegonium

83. What is the difference between a seed and an ovule?

84. What is the difference between a stamen and a sporophyll?

85. What is the difference between a sporangium and an ovule?

86. What is the difference between an integument and a sporangium?

87. When pine seeds germinate, what structures of the embryo become prominently enlarged?

88. How can you distinguish cotyledons and foliage leaves?

89. What happens to the tissue of the gametophyte which forms part of the seed?

90. What are the principle differences between pines and ferns in method and structures of reproduction?

The structure of ovulate cones in conifers is as variable as the habit of the plants themselves. In this laboratory, pine was used only as illustrative material. Other genera may produce seeds in one year or less. The procedure in all cases is similar. In the cycads and *Gingko*, swimming sperms are released into the archegonial chamber. In the conifers, the sperm cell does not swim but is carried to the egg by growth of the pollen tube.

Additional Summary Questions and Review

The following summary and activities may be considered as a post-laboratory summary. You may want to consider outside resource materials in addition to your class notes and text.

Summary

Conifers are represented by plants ranging in size from ________________ to ________________. The usual habit of the trees is ______________________________. The leaves range in size from ______________________ to ______________________ and may be arranged in several ways, viz: ______________________________.

Some conifers retain leaves for several years and are commonly referred to as evergreen. Others lose their leaves annually and are said to be ________________________________. Where branches are of distinct kinds, rate of growth often distinguishes them. Slow growing branches or branches of limited growth are called ______________________________. In pine, these branches of limited growth may produce a number of leaves characteristic of the species. This number varies from ______________ to ______________ in the several species.

Reproductive branches are in the form of cones or strobili. There are two kinds of cones called ____________________________ and ____________________________. These branches are usually produced in the spring and occupy a ____________________________ position on the stems.

The clustered strobili are composed of leaf like structures called ______________________________ and bear sporangia on the ______________________________ side. Spore mother cells produce many microspores which germinate within the sporangium to form ______________________________.

Other strobili are structurally complex and seem to consist of scales in the axils of ______________ ______________. These scales may be comparable to the vegetative ____________________________ of *Pinus*. If this is true, the ovulate strobilus is comparable to the cluster of staminate strobili. Megasporangia enclosed partially by an integument are called ______________________________. In the megasporangium, ________________________ (how many?) spores are produced from ________________________ (how many?) spore mother cell(s). ________________________ (how many per ovule?) megaspore(s) germinate within the ovule to form the ____________________________. Eggs are produced in ____________________________ at the ____________________________ end of the sporangium.

Sperm cells are produced in the ________________________________ and reach the eggs by ________________________. The product of fertilization is called the ________________________. This diploid cell germinates to form the ________________________________, which consists of ________________________________. The food for growth of the new sporophyte is derived from the ______________________________. After a period of growth, the young sporophyte becomes dormant, and the structure containing it is shed from the cone. The ripened ovule containing the gametophyte and the embryo is called a ________________________________. The embryo on germination produces ________________________________.

The process by which the immature male gametophytes are carried to the ovules is called ____________________. The process of union of egg and sperm is called ____________________.

Further Research

If you are interested in exploring gymnosperm variations further, you may wish to investigate other genera. Observe material from other conifer species as available.

With the guidance of your instructor, study prepared slides of *Ephedra* and write a short research paper on *Ephedra* using library materials. In the paper, answer the question, how might *Ephedra* be classified?

Resources

Anderson, E. "Cone and Seed Studies in Norway Spruce (*Picea abies*)," *Studies Forestry Suecica*. V 23, 1965.

Kumlien, L. L. *The Friendly Evergreens*. Rinehart: New York, 1946.

Schulman, Edmund, and W. Robert Moore. "Bristlecone Pine, the Oldest Known Living Thing," *National Geographic*. V 113, 1958.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

- cambium
- conifer
- cork
- cortex
- cycad
- gymnosperm
- integument
- micropyle
- ovule
- periderm
- pollen
- resin canal
- secretory cell
- short shoot
- sieve cell
- spring or summer wood
- stamen
- tracheid

Review

21. Introduction to the Gymnosperms

Name______________________________

Date_______________________________

Matching

______ 1. xylem	A. phloem
______ 2. integument	B. periderm
______ 3. sieve cell	C. tracheid
______ 4. cork	D. microsporophyll
______ 5. pollen	E. seed coat

Multiple Choice

______ 6. Which group of gymnosperms continues to employ a flagellated sperm in reproduction?

a) conifers
b) *Ephedra*
c) cycads
d) *Gingko*

______ 7. Cones of gymnosperms are modified

a) roots
b) stems
c) leaves
d) flowers

______ 8. The largest group of gymnosperms are the

a) conifers
b) *Ephedra*
c) cycads
d) *Gingko*

______ 9. The word gymnosperm literally means

a) naked seed
b) covered seed
c) athletic seed
d) fern-like seed

______ 10. The megasporangium with its integument is called a(n)

a) integument
b) ovule
c) stamen
d) *Zamia*

Essay

1. What is a cone? Describe its structure

2. Describe the life cycle of a pine tree

Answers Found: p 235 - #9; p 237 - #2 & 10; p 238 - #5; p 239 - #6; p 240 - #8; p 242 - #1, 3, & 4; p 247 - #7. Essay #1: pp 247 & 248; Essay #2: pp 247 - 251.

Animal Growth & Behavior

22. Discussion

Hybridomas and Monoclonal Antibodies

When you have completed this discussion you should be able to:

1. Describe how the vertebrate immune system works, including the production of antibodies.
2. Explain self, nonself, and tolerance identification by human lymphocytes.
3. Detail the human autoimmune response, and allergies or diseases associated with its failure.
4. Explain the importance of cell tissue culture in medical research.
5. Describe hybridomas and monoclonal antibodies and their contribution to immunology.

The Immune System

The immune system is key to the survival of vertebrates, for without it death from infection would be certain. The components of the immune defense network maintain constant surveillance against foreign invaders. The system is able to recognize an almost limitless variety of foreign cells and substances and to mobilize against them. Special cells in the immune system carry the memory of each infection so that a re-exposure is countered almost immediately. The system functions to recognize, selectively eliminate, and remember each type of foreign invader. It is now recognized as a major body system, along with the circulatory, nervous, muscular, skeletal, digestive, endocrine, excretory, and reproductive systems.

Immune Responses

There are two forms of immune response in the human body. The cellular immune response, based on special cells sensitive to foreign cells and molecules, generally fights against larger foreign substances such as cancer cells, fungi, and multicellular parasites. The humoral immune response, based on antibodies present in blood and other body fluids, specializes against bacteria and viruses which have not yet invaded body cells. The two immune responses can act in tandem in the blood or lymphatic systems of the body.

Lymphocytes

The primary cells of the immune system are small white blood cells called lymphocytes. Lymphocytes, like other blood cells, are produced in ancestral, or stem, cells in bone marrow. Two types of lymphocytes have been identified in humans, *B* and *T*. The *B* lymphocytes complete their maturation within bone marrow. The *T* lymphocytes migrate from bone marrow to the thymus, where they are further differentiated. *B* and *T* lymphocytes are similar in size and appearance, but their immunological roles are different.

B Lymphocytes

B lymphocytes manufacture antibodies. Antibodies are immunoglobulin proteins produced in response to specific antigens. Their functions include neutralizing toxins, agglutinating bacteria or cells, and precipitating soluble antigens. Antigens are any protein or carbohydrate which stimulates an immune response and the production of antibodies. When most individuals respond the same negative way to a substance, it is called a disease; when only a few experience an immune response, it is called an allergy.

Sir Macfarlane Burnet, an Australian microbiologist, developed the clonal selection theory of immunology which generally explains the basic mode of *B* lymphocyte action. According to the clonal selection theory, as each *B* cell matures in the bone marrow, it becomes commited to the synthesis of antibodies which recognize a specific antigen, or molecular pattern. The molecular pattern which the antibody recognizes on an antigen is called the epitope. In the simplest case, the descendants of each such cell retain the same specificity, and they thus form a clone of immunologically identical cells.

A *B* cell's antibodies stay bound to the cell membrane, where they are displayed on the surface as receptor molecules. When and if an antigen binds to an antibody in the membrane, the cell is stimulated to proliferate. This is called the process of clonal selection. Many different clones can respond to a single infection. The antigen markers can be very small patterns of molecular structure, and a single virus or bacterium carries many such patterns.

Complement Proteins

Antibody molecules do not directly destroy a foreign invader; they mark it for destruction by other defense mechanisms. One of these mechanisms is comprised of complement proteins, or enzymes. Complement proteins consist of at least eleven different proteins circulating in the body. Their attacks on invaders are triggered by antibodies attaching themselves to the cell membranes of bacteria, protozoa, or tumor cells.

When stirred into action by the binding of immunoglobulin to antigen, complement proteins attach to the cell membrane and cause it to burst. Because they are not altered or used up by the process, these complement proteins can be considered a special type of enzyme.

A second defense mechanism consists of phagocytic cells moving in the circulatory system which recognize and consume antibody marked substances. A third way antibodies work in defense is to coat or combine with an invader and interfere with one of its vital functions. No further action by complement proteins or phagocytes is necessary, since the act of binding with the particle de-natures it. For example, when an antibody combines with a virus it prevents it from invading a host cell.

Antibody Diversity

A virus or bacterium has many different epitopes, or centers for antibody attachment. Antibodies can be formed for each different epitope on the invading organism, and although one antibody is sufficient to control an invader, the body usually produces a polyclonal, or more than one antibody, reaction. Each different antibody comes from a separate *B* lymphocyte which produces a unique antibody.

One mouse can produce an estimated billion different antibodies. A single antigen may elicit several thousand different antibodies. Antiserum effective against even a single antigen is almost impossible to duplicate. The use of conventional immune serum, because of polyclonal responses, can have unforseen side effects. Minor contaminants in the serum may cause untoward allergic reactions. Even highly specific antibodies can exhibit heterogeneity and cross reactivity, and they are available only in limited quantities.

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T Lymphocytes

It is more difficult to classify and to describe *T* cells than *B* cells. Most *T* cells do not recognize freely circulating antigens, and they respond to an antigen on a cell surface only under particular conditions. The foreign invader must be in the process of invading a host cell before there is a *T* cell response. For an immune response to occur, the antigen receptor on the surface of a *T* cell must simultaneously recognize the antigen and the host cell protein.

There are four types of *T* lymphocytes and four *T* responses. The cytotoxic, or killer *T* cells destroy targeted invader cells without further assistance. The other three types of *T* cells variously modulate the immune response by secreting messenger proteins or by directly contacting cells under attack. One of these three is referred to as an inducer *T* cell because it triggers the maturation of *T* lymphocytes from precursor forms into functionally distinct cells. The so-called helper *T* cells, once they recognize an antigen, stimulate other components of the immune system, including *B* cells and other *T* cells specific for the same antigen. The final type, suppressor *T* cells, diminish the activities of all other lymphocytes, thus permitting the body to return to a normal state.

While the term helper *T* cell implies a subsidiary role in the immune system, the opposite in fact may be true. Some believe that helper *T* cells might be master switches for the entire immune system. The suppressor *T* cells might be equally important, for they provide the negative feedback vital to making the immune response self-limiting.

Self, Nonself, and Tolerance

Since *B* and *T* lymphocytes react to foreign invaders, a critical question is how they recognize foreign substances. The English immunologist Peter B. Medawar in 1953 attempted to answer this question by taking lymphoid cells from one variety of adult mouse and injecting them into newborn mice of another variety. At eight to ten weeks of age he grafted skin from the original adult mice onto the younger mice, as well as skin from a third strain of mice. The younger mice accepted the skin of the original adult mice as their own but rejected the skin of the third mouse strain. These results showed that immunological tolerance, acceptance of a substance as part of self, was induced by exposure to the antigen early in the development of the newborn mice.

If a substance ceases to be produced in the body, the body (via the lymphocytes) ceases to regard the substance as part of itself. This reaction is called clonal deletion. If the body resumes production of the substance, the lymphocytes react to the substance as foreign and move against it, eliciting a full-fledged autoimmune, or self allergy, disease.

Edward Triplett in 1962 at the University of California removed the pituitary glands of frog embryos and stored them under the skin of other young frog embryos. Later, when the original owners had matured, Triplett returned their pituitaries to them, whereupon the glands were rejected. If any piece of the original pituitary had been left in place, the returned gland was not rejected. The immune system's knowledge of self and nonself is an important key to an organism's defense system. Edward Triplett studied self-nonself identification and organ transplant rejection in frogs. Today medical researchers are studying human organ transplants and considering methods to suppress immune system attacks until a new organ has established itself in the recipient's body.

Immune System Disorders

Autoimmune Diseases

Sometimes the immune system's recognition of self and nonself fails, and lymphocytes react against self. Once stimulated into production, they can perpetuate and wreck considerable damage upon the body. Among the autoimmune (self-directed) diseases of humans, in which components of the body are attacked by its own immune system, are juvenile-onset diabetes, lupus erythematosus, multiple sclerosis, myasthenia gravis, rheumatic fever, rheumatoid arthritis, several types of anemia, and ulcerative colitis.

Quite simply, different parts of the body are targeted by lymphocytes for destruction. In lupus erythematosus antibodies attack the skin, in myasthenia gravis antibodies attack neuromuscular junctions, multiple sclerosis attacks are centered in the brain and spinal column, in rheumatic fever antibodies attack the heart, in rheumatoid arthritis the bones and joints are under seige, and in ulcerative colitis it is the colon. Autoimmune diseases may attack almost every known part of the body, from muscles to the thyroid. Doctors can mask the symptoms of autoimmune diseases, but there is no cure, unless the body somehow cures itself. That seldom has been known to happen.

The Rh Factor

Hemolytic anemia, which results from Rh incompatibility between mother and child, can be a serious problem for newborn babies. The human baby acquires antibodies from its mother during its last month in the uterus. Most of these antibodies are beneficial. The exception is the Rh factor, which is a genetically determined substance found on the surface of red blood cells. If an Rh negative woman carries a child who is Rh positive, her blood stream will form antibodies against the blood of the Rh positive child. The first born child generally escapes without serious damage, but the antibodies against Rh positive blood persist in the mother. In each subsequent pregnancy the mother's immune system attacks more vigorously the fetal bloodstream, and the mother's blood can seriously harm or kill the nearly full-term child.

Now that the causes of hemolytic anemia are known, Rh disease can be suppressed by injecting the mother, within seventy-two hours of any abortion or live birth, with antibodies against the fetal Rh red blood cells still in her system. This destroys the remnant fetal cells and prevents the triggering of antibody production. Subsequent pregnancies will be at no greater risk than the first.

Another immune system problem for some women is the build up of antibodies against their husband's sperm. These react to the sperm as foreign invaders and destroy them before conception can occur. Since the reaction is cumulative, the couple may have one or two children before the problem becomes obvious.

Allergies

People are routinely exposed to environmental antigens, such as pollen, food, animal parts, or dust. In some people, there is an allergic reaction caused by inhaling, eating, or touching foreign substances. Although the antigen itself may present no danger to the individual, an excessive immune response does present a problem, either in terms of discomfort or, in extreme cases, death.

An allergic response builds over time. The antigen causes a response in the immune system. Lymphocyte memory cells are produced, and the next time the antigen is encountered, the antibody response is much stronger. Eventually the antigen can be met with such a strong antibody response that the body may fatally overreact. In fatal bee stings the reaction is generalized throughout the body. Blood vessels dilate (under the influence of histamine) in an inflammatory response, others constrict, blood is misdirected, shock follows, and then death.

Other well known allergic reactions include hay fever where airborne pollens or dust cause reactions in nasal passages, and asthma where the reaction is centered in the lungs. Food allergies can produce symptoms that affect the intestines or produce skin rashes. In serious cases, food substances may be absorbed into the blood steam and cause a general reaction, at times life threatening.

Antihistamines, which counteract the histamine effect, suppress some of the symptoms of an allergic response. Steroid hormones are also effective in some severe cases. Again, as with autoimmune diseases, medical researchers are still at the point of treating the symptoms, not effecting a cure or preventing the syndrome.

Cancer, Viruses, and the Immune System

When body cells mutate into something other than the role they were genetically destined to play, as in the case of cancer, it is appropriate that the body's defense system mount an all out attack against these deviant cells. The transformation of a normal cell in to a cancer cell may begin in many ways, and such triggering mechanisms are under medical study throughout the world. We know that nuclear changes can be induced by infection with viruses, particularly the retroviruses. Retroviruses are mostly oncogenic, or tumor inducing viruses. The retroviruses are RNA viruses which are dependent upon cellular DNA synthesis for growth. When they transform a cell, the transformation is genetically stable, so that a single cell will produce a clone of cancer cells. This yields the tumor.

Cell membrane changes also occur in cancer cells. Cell membrane changes transform the cancer cell into an invasive cell with no restraints on growth. Cancer tumors are malignant, in that they invade surrounding tissues and spread to other parts of the body. This metastasis occurs in two stages. First, cancer cells invade surrounding tissues, and then they enter the circulatory system. The sequence is the same for most cancers, including leukemia, which begins in bone marrow, although some cancers act much faster than others.

Cell membrane changes in cancer cells also yield a positive side effect. Once the cell membrane has changed, the immune system normally is able to identify these cells as nonself, form antibodies against them, and destroy them. As early as 1908 the German microbiologist Paul Ehrlich suggested that cancer might in fact be a very common occurrence but that most people possessed an immune system which routinely is able to detect and destroy deviant cells. This logically suggests that all cancers fundamentally represent failures of the immune system.

Very recently Leo Sachs of the Weizmann institute of Science in Israel demonstrated that it is even possible to reverse the malignancy of a cell by altering its environment. The medical applications of this discovery are potentially considerable.

AIDS

AIDS, or acquired immune deficiency syndrome, is a retrovirus caused fatal disease. Like most retroviruses, the AIDS virus changes the nuclear structure of a cell. Often AIDS victims die of a previously rare cancer, Kaposi's sarcoma, a cancer of blood vessel linings. Also, like many other viruses in this group, the AIDS virus produces a lasting depression of the immune response. It destroys *T* lymphocytes. As a consequence, AIDS patients may eventually die from any number of minor and normally not fatal infections.

The depression of the immune system in AIDS has parallels in other viral infections. For example, Clemens von Pirquet in 1908 noted that measles temporarily weakens immunity. Von Pirquet found that people who have been exposed to tuberculosis, and normally show positive with a tuberculin skin test, lose that skin reactivity for a period of time after having had measles. Another example is the common and generally correct belief that a bout with the flu often leaves an individual in a weakened condition and susceptible to other communicable diseases. However, retroviruses differ from other groups of viruses in that they produce a permanent depression of the immune system.

AIDS not only depresses the immune system, it eventually destroys it. AIDS viruses, like some other viral groups, initially fight off the immune system by frequently mutating, causing antigen-specific immune responses to miss the mark. This is called anti-genetic drift, and it is the reason why antibodies extracted from the blood of AIDS patients are ineffective against the virus.

Cell Tissue Culture

Some readers may be asking how the preceding pages relate to the title of this discussion, "Hybridomas and Monoclonal Antibodies." It is a good question, but before attempting an explicit answer, it is necessary to provide one more facet of the story, the matter of cell tissue culture. And this story can begin with an Englishman, H. B. Wilson, who is 1907 decided to take a leisurely lunch after a full morning in his laboratory. At the time he was studying sponges, and, wanting to perform experiments on individual cells, he forced a sponge through cheese cloth, which caused the sponge cells to separate. Upon his return from the leisurely lunch, something very startling had occurred. The sponge had reformed into a complete organism.

Wilson repeated the experiment several times, including separating two types of sponges and mixing the two cell types together. He found that the sponge cells sorted themselves out and still reformed two types of sponges. These experiments led Wilson to a lifetime of sponge studies and caused other scientists to conclude that sponges are colonial creatures, not true multicellular organisms. Wilson's experiments were simply noted as an oddity peculiar to sponges and the subject generally dropped by the scientific community.

Even though Wilson's experiments did not receive the attention they merited, they did stimulate some scientists to wonder how cells of true multicellular organisms could be induced to grow separately in live cultures. Wilson had mechanically dissociated sponge cells. Finally, in 1916, Rous and Jones successfully dissociated cells chemically. They used partially purified trypsin powder to chemically dissociate cells growing from tissue fragments explanted in plasma clots. They demonstrated that the freed cells could then be subcultured in fresh plasma. It was the first time that anyone had successfully grown cells of a true multicellular organism outside that organism.

In Vitro Systems

Ross Harrison in 1929 was able to standardize *in vitro* tissue culture, that is, he was able to grow various tissues which reproduced and lived outside source organisms. Harrison still later pioneered work with polio viruses by growing the virus *in vitro* in monkey kidney cells. By the 1940's investigators were separating and culturing many types of tissues through various *in vitro* techniques. *In vitro* culture systems proved to be a turning point in virology studies, for they provided a means to study viruses outside a living organism.

The first cell lines of classic human malignancies were being cultured by the end of WWII. *In vitro* systems allow study of a cell isolated from other influences, cellular or otherwise. They permit control of the physical environment (pH, temperature, pressure, etc.) in which a cell grows. They also offer the opportunity to clone cells, while providing a measure of genetic control and uniformity of the population under study. *In vitro* cell systems, in addition to cancer, viral, and bacterial studies, can be used in medical research for chromosomal studies, cell fusion procedures, and investigations into the molecular bases of genetic disorders.

Cells growing *in vitro* have special culture requirements, as they are extremely sensitive and perishable. For example, culture vessels must be non-toxic, and only de-mineralized, de-ionized, distilled water can be used. In addition, cells normally cannot proliferate unless they can attach to a substratum, which in some cases can be the plastic or glass container in which they are being cultured. An exception is cancer cells which do not require attachments. The medium must be carefully defined, so that any changes are attributable to the culture alone. Despite all these, and still other necessary precautions, there is a high frequency of abnormal cells, due to the unnatural environment in *in vitro* systems. This is characteristic of cells in culture.

Fibroblastic and Epithelial Cells

There are two basic growth patterns in multicellular, proliferating cells. In one type of cell pattern, fibroblastic, the cells grow oriented to each other, normally in a single layer due to a factor called migration inhibition, which discourages them from growing on top of one another. Due to contact inhibition, they stop growing when they have no more room to spread in the culture vessel.

The other type, epithelial cells, maintain strong contacts, called junctions, between cells. They thus tend to grow in packets or clusters until they fill the entire culture vessel.

Culture Patterns

Cellular aggregation patterns can be altered by factors other than whether the cell's growth pattern is fibroblastic or epithelial. Age, health, and medium characteristics all play a role. The normal adhesive tendencies of cells can be inhibited mechanically by maintaining the culture in a rotating flask. Aggregation also can be affected chemically by changing the concentration of solutions used as nutrients for a cell culture.

Individual cells proliferate at a rate similar to what might have occurred in the source organism. For example, in a mixed culture, brain cells do not proliferate, bone and blood cells increase slowly, but fibroblasts, because of their wound healing properties, increase rapidly, just as they do in the source organism. Therefore, as a mixed culture continues through the generations, certain types of cells become increasingly less common. Neural cells do not replicate and eventually disappear, whereas neural connector cells called glial cells undergo mitosis and maintain a presence within a mixed culture.

When a cell turns cancerous, culture patterns change radically. Cancerous cells do not depend on attachment to a substratum, they clump together, and their growth is unchecked. In laboratory settings they spread to the outer limits of the culture's nutrient solution.

Characteristics of culture pattern will be used in the following laboratory to distinguish cell types.

The Aging Process

Hayflick and Morehead, in the late 1950's and early 1960's, monitored cell reproductive processes by culturing human fetal lung tissue. They discovered that cells in culture have a finite life span. After fifty doublings, human fetal lung tissue stops proliferation. The growth curve becomes flat and eventually turns down. If hydrocortisone is added to the culture, proliferation can be expanded, but not indefinitely. The culture ages and, in essence, dies. However, if the human fetal lung tissue cells had been cancerous, they would have continued to grow endlessly, as if they were immortal.

Hybridoma and Monoclonal Antibodies

Hybridomas

An important breakthrough in cell culture was achieved in the mid-1970's by Cesar Milstein, an Argentinean, and Georges Köhler, a Swiss, conducting research in Cambridge, England. Milstein and Köhler, in common with other researchers working on antibodies at the time, had difficulties because of the body's production of a complex mixture of antibodies, making it almost impossible to separate specific antibodies for study. Milstein and Köhler searched for a method to produce, isolate, and purify specific antibodies.

Milstein and Köhler applied the knowledge that cancer cells are immortal in culture. They separated and cultured myelomas, a bone marrow cancer, which they hoped might be compatible with the lymphocytes which produce the antibodies they were studying. Using a newly developed technique, they fused the cancerous myeloma cells with the target lymphocytes. The result was immortal cells which produce antibodies even in the absence of antigens. They were able to fuse these two different cells because of the discovery that polyethylene glycol, abbreviated PEG, causes cell membranes to lose their individual identity markers. They had combined a normal lymphocyte with a myeloma and had produced a hybrid immortal cell which makes specific antibodies. They termed this new cell a hybridoma. It was a discovery worthy of the Nobel Prize. Now specific antibodies can be produced in sufficient quantities for both research and medical applications.

Cloning Hybridomas

Cloning hybridomas is done as follows. An animal is inoculated with an antigen to trigger lymphocyte and antibody production. Later, a blood sample is taken and a mixture of lymphocytes is extracted from the blood. Next, the lymphocytes are fused with myeloma cells from a single tumor. The mixture of hybridomas is then allowed to proliferate (clone) in separate little wells (tiny petri dishes). Each dish is tested for antigen-antibody reactions. This sifting process is greatly facilitated today by computer-aided chemical analysis, whereas Milstein and Köhler culled their tiny dishes manually.

Positive dishes are separated from the rest. The dishes are again cloned and tested further for specific reactions. From literally thousands of little dishes researchers are fortunate to find a single, pure hybridoma culture which produces a desired antibody. It is similar to searching for the proverbial needle in a haystack. Rare finds are those which produce monoclonal antibodies, or uniform antibodies from a single clone of cells. Once isolated the monoclonal antibody producing hybridoma can be multiplied indefinitely by cell tissue culture to supply the world's needs for that particular antibody. Such potentially profitable isolations are now patented.

Monoclonal Antibodies

Monoclonal antibodies are ideal for the study of antibody chemistry, and with sufficient volumes of antibody now available, they are used to extend our knowledge in other areas, such as cell membrane specificity and tissue typing. Researchers now are able to explore the possibility of passive immunization, in which people would be vaccinated with a specific antibody rather than with an antigen, as now done, which causes a patient to develop their own antibodies. Until the monoclonal antibody technique, large quantities of pure, specific antibodies were unavailable for this procedure.

The medical benefits of monoclonal antibody production are just now beginning to accrue, and the potentialities are enormous. Researchers now have volumes of pure antibodies which yield consistent results.

Cell Lines

In the United States, the Hybridoma Cell Bank, supported by the National Institute of Allergy and Infectious Diseases, began operation in October 1980. The principal aim of the bank is to collect hybridoma cell lines secreting monoclonal antibodies. Groups and individuals may deposit cell lines for preservation and storage. Researchers from around the world may then order the cell lines they wish to investigate. The cell bank checks cultures for contamination with mycoplasmas and bacteria and for species identification. It also performs analytical isoelectric focusing in flat-bed thin layer agarose gels on the cultures submitted.

The American Type Culture Collection

The Hybridoma Cell Bank is only one section of the American Type Culture Collection (ATCC) located in Bethesda, Maryland. It was the first approved international depository under the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures. Also, American and European patent organizations consider the ATCC as a legal depository for patent verification.

At present, one can only speculate as to what the eventual industrial, medical, and research impact of hybridomas and monoclonal antibodies might be. It is, however, certain to be substantial.

Resources

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Terminology

Students should understand the following terms and concepts prior to taking the unit review.

- AIDS
- allergy
- antibody
- antigen
- autoimmune disease
- cancer
- cell tissue culture
- complement protein
- epithelial cells
- epitope
- fibroblastic cells
- hybridoma
- immune system
- *in vitro*
- lymphocyte
- monoclonal
- polyclonal

Review

22. Hybridomas and Monoclonal Antibodies

Name ______________________________

Date ______________________________

Multiple choice

______ 1. *T* lymphocytes mature in the

a) marrow of bones
b) blood
c) thymus
d) thyroid

______ 2. The humoral immune response acts primarily against

a) fungi
b) bacteria
c) cancer cells
d) intracellular viral infections

______ 3. The spreading of malignant cells to other parts of the body is known as

a) metastasis
b) myeloma
c) chemotherapy
d) sarcoma

______ 4. They manufacture antibodies.

a) *T* lymphocytes
b) Rh factors
c) complement proteins
d) *B* lymphocytes

______ 5. An epitope is

a) a center or molecular pattern for antibody attachment
b) a group of complement proteins
c) supperssor *T* cells at work
d) an area or center for cryogenic preservation

______ 6. These *T* lymphocytes might be the master switches for the entire immune system.

a) killer *T* cells
b) inducer *T* cells
c) suppressor *T* cells
d) helper *T* cells

______ 7. An immune response elicited against self is

a) clonal deletion
b) clonal tolerance
c) clonal selection
d) clonally impossible

______ 8. This is an autoimmune disease.

a) some types of allergy
b) AIDS
c) asthma
d) some types of anemia

______ 9. These cells grow rapidly but in a single layer when in cell tissue cultures.

a) epithelial cells
b) glial cells
c) fibroblastic cells
d) cancer cells

______ 10. Cells which are a cross between a normal cell from a multicellular organism and a cancerous cell are called

a) epitopes
b) hybridomas
c) monoclonal antibodies
d) myelomas

Short answer:

11. What is the basic premise of the clonal selection theory? ______

12. What is the relationship between the immune defense system and cancer? ______

13. In discussing the body's immune system, what do we mean by self and nonself? ______

14. What are the differences between *B* and *T* lymphocytes?

15. What are hybridomas and monoclonal antibodies?

Answers Found: p 257 - #2 & 4; p 258 - #5 & 11; p 259 - #1, 6, 7, & 8; p 261 - #3 & 5; p 262 - #9; p 263 - #10; Short Answer: #11 - p 257; #12 - p 261; #13 - p 259; #14 - p 257, 258, & 259; #15 - p 263 & 264.

23. Laboratory

Creating a Heterokaryon

When you have completed this laboratory you should be able to:

1. Demonstrate asceptic technique in cell tissue culture.
2. Fuse two different cell lines with polyethylene glycol.
3. Create a heterokaryon.
4. Identify and describe the new cells.

Purpose

Two stages are required for cells to fuse. In the first stage the cell membranes integrate, and the cellular cytoplasms fuse. A cell in this first stage of fusion is called a heterokaryon. Many heterokaryons exist naturally, such as binucleate and coenocytic algae. In the second stage the two nuclei within the cytoplasm fuse. After this stage of complete fusion the cell is called a hybridoma, as discussed in the last unit.

In this six day laboratory you will force cells to physically fuse, and you will observe this through the first stage of fusion. You will create a new living organism, a heterokaryon. You will be able to observe it functioning and reproducing. If you were to maintain the culture over several weeks, you might see some of the heterokaryon cells become true hybrids or hybridomas.

Two cell lines have been chosen for this experiment which are not virulent to humans. However, it would be prudent to exercise care so that neither cell lines, laboratory, nor experimenters are accidentally contaminated. The first cell line used for this fusion exercise is the same mouse myeloma used by Drs. Georges Köhler and Cesar Milstein during their hybridoma experiments in Cambridge, England. It is a non-secretory myeloma, or primary tumor of mouse bone marrow. The other cell line also comes from a mouse. It is a non-cancerous spontaneous tumor, called a neuroblastoma, taken from a mouse brain. It secretes acetylcholinesterase, together with enzymes for the synthesis of the neurotransmitters, choline acetylase and tyrosine hydroxylase.

In this laboratory you will fuse two morphologically distinct cell lines, brain and bone. You will study the parent cell lines, subculture them, fuse them, prepare slides of both pure and fused cultures for staining, and cytologically stain both parent and fused cell lines. You will then study the stained cells to see if fusion between the brain and bone cell lines did indeed occur.

Pre-lab

Supplies needed:

Equipment

inverted microscope
microscope
centrifuge
2 centrifuge tubes, 15 ml.
37° C. incubator
styrofoam cup
staining kit (5 vials)
6 slides
2 petri dishes

4 sterile 1 ml. pipets
8 sterile 5 ml. pipets
10 sterile 10 ml. pipets
non-sterile pipets
timer or watch
3 sterile 25 ml. flasks
6 sterile Leighton tubes with Morton closures
6 sterile Leighton flying coverslips

6 large coverslips, #1, 24 × 50 mm.
curved forceps
probe
2 pi-pumps, 1 for 1 & 2 ml. pipet size and 1 for 5 & 10 ml. pipet size, or 1 Pipet Aid (electric)
bunsen burner
marking pen

Materials

confluent NIE 115 (Clone Neuro-2a from tumor line C1300) cells. mouse brain tumor
confluent NS 1 (P3/NS1/1-Ag4-1) cells. mouse cancerous bone marrow
Dulbecco's modified Eagle. or minimum essential medium. 10% fetal bovine solution with penicillin and streptomycin (DMEM 10% FBS 1/2% PS)
serum free DMEM (SF DMEM)
50% polyethylene glycol (PEG) in SF DMEM. with a molecular weight of 4000
phosphate buffered solution (PBS) with a pH of 7.4
permount
toothpicks
70% alcohol
hand cream

Optional: sterile 0.2 μm. beads. 2 colors

Special Preparations

1) Cell lines may be obtained from local universities or from the American Type Culture Collection (see Appendix B). Plan the laboratory well in advance. but. if possible. avoid contamination during handling and subculturing by arranging to have cultures for each laboratory group delivered just two or three days in advance of laboratory start. Each 25 ml. flask should contain 5 ml. of cell suspension. This delivery schedule allows time for the cells to grow into confluency in the culture flasks (to cover surfaces of flask under liquid one cell layer deep). At the same time. not enough time has elapsed to require a media change. Upon arrival. immediately incubate culture flasks at 37° C.

If you do not wish to purchase individual flasks for each laboratory group. have the cell cultures delivered one week in advance of laboratory start. When the cells have grown to confluence (two to three days). subculture them. per the steps delineated in hour two. so that every laboratory group will have 5 ml. of cell suspension of each of the two parent cell lines in separate 25 ml. cell tissue culture flasks. Subculturing one flask provides cells for many groups.

2) Prepare staining kits for each student group by preparing sets of labelled 30 ml. plastic vials containing the following stains and reagents: 40% methanol. isopropyl alcohol. distilled water. freshly prepared Giemsa stain. and xylene.

To make Giemsa stain dissolve 0.5 gram Giemsa powder in 33 ml. glycerin (dissolution may require one to two hours). Next. add 33 ml. acetone-free absolute methyl alcohol. This is a stock Giemsa stain solution. To prepare for students. at the earliest 24 hours before use. dilute each ml. of stock by adding 20 ml. distilled water.

3) As to quantities. plan for each student group to use 20 ml. DMEM. 11 ml. SF DMEM. at least 85 ml. PBS. and 1 ml. PEG. Provide 1 ml. of each color marker bead to each student group. if it is decided to use them. The solutions are available from commercial suppliers. Universities also can be a source of biological materials.

4) A 100 ml. bottle of permount is sufficient for several years of this exercise. It would be best for the instructor to carefully dispense the permount on toothpicks as it is being used. because it hardens very quickly upon exposure to air.

5) To set up student equipment. have two or three student groups share one centrifuge. When the 37° C. water bath is required. prepare one for the entire class.

6) Provide quantities of 70% alcohol for cleaning work surfaces. particularly during hours two and three of this laboratory. Because alcohol can dry the skin. students appreciate having hand cream available.

7) Pi-pumps and Pipet Aids are described in most biological supply catalogues. Both are excellent pipetting devices.

8) Hand the students coverslips and Leighton tubes which have been sterilized as a unit.

9) Use of marker beads is **optional** because of cost. Although students can identify the two cell types without marker beads, they do dramatically highlight the separate lines and the fact of fusion. Beads especially designed for this work are called dyed polystyrene microspheres with 2.5% solids-latex. If used, add 1 ml. 0.2 μm. beads to each of the two 25 ml. culture flasks. Use a separate color for each of the parent cell lines four to six hours before attempting the fusion step during hour three. The marker beads will be incorporated by the cells and provide an identifiable internal marker when viewed with a light microscope.

Time Required

The creating a heterokaryon laboratory requires six classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the following supplies: non-sterile pipets, pi-pumps or Pipet Aid, inverted microscope, and confluent cultures of NIE 115 mouse brain tumor cells and NS 1 mouse cancerous bone marrow cells.

During this laboratory hour you will study the morphology of both parent cell lines. You also will practice using the inverted microscope and practice the art of pipetting with pi-pumps or other pipetting devices, as well as review asceptic technique.

Steps

A. Review the following cell tissue culture asceptic techniques:

1) Prepare the work area by wiping it with 70% alcohol. Also rinse your hands with alcohol.

2) Do not uncover any sterile glassware such as flasks, pipets, or petri dishes, until required. Never leave them uncovered. Remember, sterile pipets do not have to be flamed, and hot pipets kill cells.

3) Do not hold any opening straight up to the air. Microorganisms fall straight down, so hold openings to the side. Flame the lips of such containers both before and after introducing a pipet and/or closing it.

4) **Never** pipet by mouth. Use a pi-pump or similar device. Even though sterile pipets are plugged, mycoplasmas from the mouth can still creep through the cotton, and mycoplasma contamination is a major source of tissue culture contamination.

5) Use a sterile pipet **each time** you draw from a bottle. Once the pipet has been exposed, the chance that it has been contaminated is too great to risk reuse. This heavy use of pipets is essential to maintaining pure cell lines.

6) Pipetting and other techniques in this laboratory need to be performed as rapidly as possible to minimize opportunities for contamination.

7) There should be no talking, singing, or whistling during the execution of sterile procedures.

8) The success of this laboratory greatly depends on clean and precise execution.

B. Practice pipetting exact volumes using non-sterile pipets and plain water. Accustom yourself to pi-pumps, or similar pipetting devices, and how they operate. The more facile you become now with pipetting technique, the easier it will be to accomplish cell fusion successfully without contamination, and in the allotted laboratory hour.

C. Study the structure and function of an inverted microscope.

D. Place the NIE 115 cell tissue culture flask on the stage of the inverted microscope and bring the cells into focus, first on low and then on high power.

1. Describe how the cells physically orient to each other.

2. Are they congregated in a specific area of the flask? If so, where?

3. Select, draw, and label three typical NIE 115 cells in the space provided below. Be very careful to determine the proportion of cytoplasm to nucleus, and note any irregularities in the cell membranes.

E. Observe the NS 1 cell tissue culture flask under the inverted microscope, first on low and then on high power.

4. Describe how the cells physically orient to each other.

5. Are they congregated in a specific area of the flask? If so, where? ______________________________

6. Select, draw, and label three typical NS 1 cells in the space provided below. Be very careful to determine the proportion of cytoplasm to nucleus, and note any irregularities in the cell membranes.

7. How are NIE 115 and NS 1 cultures different? Note differences in structure which should enable you to tell these two cell cultures apart.

F. Label the cell culture flasks you have observed and return them to the 37° C. incubator.

8. Why is it necessary for the incubator to be set at 37° C.? ______________________________

Procedure: Hour 2

Students need the following supplies: the labelled NIE 115 and NS 1 cell culture flasks, 2 sterile 25 ml. cell culture flasks, 4 sterile Leighton tubes with flying coverslips and Morton closures, 2 sterile 10 ml. pipets, pi-pump or other pipetting device, and 10 ml. DMEM 10% FBS 1/2% PS (hereafter referred to as DMEM).

In this laboratory hour you will study two established cell lines and then subculture them, using aseptic technique.

A. Observe again both the NIE 115 and NS 1 cultures under the inverted microscope, first under low and then under high power. Verify that the morphology and cell structures are the same as those observed in hour one.

9. Has the physical orientation of the NIE 115 cells changed since hour one? If it has changed, how has it changed?

10. Why would it (not) have changed?

11. Has the physical orientation of NS 1 cells changed since hour one? If it has changed, how has it changed?

12. Why would it (not) have changed?

B. Using aseptic technique and a 10 ml. pipet with a pipetting device, withdraw 5 ml. fresh DMEM.

C. Add the 5 ml. DMEM to the NIE 115 flask, washing the cells from the flask surface by titurating with the pipet. (**Note:** titurating is a term used to describe the sucking in and out, or up and down motion of liquid going in and out of the pipet.) Titurate **gently** until you feel certain that the cells in the flask are in the DMEM solution and not stuck to the sides of the culture flask.

D. Draw the entire mixture into the pipet. **Do not remove the pipet from the flask until you have collected the full volume of cell suspension mixture.**

E. Immediately place 5 ml. of the cell suspension into a new 25 ml. sterile flask. Then, **without touching the pipet to anything**, add 1.5 ml. to each of the 2 sterile Leighton tubes with flying coverslips and Morton closures. Dispose of the pipet and any remaining mixture.

F. Repeat the procedures of steps B through E with the NS 1 flask.

G. Label the new cultures with the proper cell line indicator, passage number (since this will be the first time you have subcultured the cell lines, this passage number will be #1), date, and your initials.

H. Observe the new cultures under the inverted microscope. Check to see that the morphology and cell structures are the same as at the beginning of the hour, thereby confirming that you have not confused cell lines.

I. Place the flasks and tubes in the 37° C. incubator. Make sure that the caps on the Leighton tubes and flasks are tight.

J. Incubate for 24 to 48 hours. Before proceeding with hour three, determine with the inverted microscope that cells have again grown into confluency.

Procedure: Hour 3

Note: If marker beads are to be used, the instructor or students, four to six hours before the start of this laboratory session, should add 1.0 ml. 0.2 μm. colored latex beads to each 25 ml. flask containing parental cell lines (different colored beads for each cell line). The 37° C. water bath, from which students will take water to fill styrofoam cups later in the hour, will need to be turned on in advance of hour start.

Students need the following supplies: inverted microscope, confluent cell cultures from hour two, centrifuge, 15 ml. sterile centrifuge tubes, 11 ml. SF DMEM, 22 ml. PBS, 1 ml. 50% PEG in SF DMEM solution, 10 ml. DMEM, 37° C. water bath, styrofoam cup, 4 sterile 1 ml. pipets, 2 sterile 5 ml. pipets, and 8 sterile 10 ml. pipets, pipetting device, 2 Leighton tubes with flying coverslips and Morton closures, and 1 sterile 25 ml. cell tissue culture flask.

Note: the instructor may wish to have available extra sterile pipets in case some students have accidents.

You will fuse two morphologically different cell lines using polyethylene glycol (PEG) during this laboratory hour.

A. Observe the parent cell line flasks under an inverted microscope.

13. Are the cells in each flask of the same type you started with during the first hour of this laboratory?

____

If the answer to question thirteen is no, you must repeat hours one and two this laboratory before you can continue. If the answer to question thirteen is yes, proceed with hour three as follows.

B. Harvest the NIE 115 flask using a sterile 10 ml. pipet with pipetting device. Harvesting of cell lines is accomplished by employing the titurating technique described during hour two.

C. Place the cell suspension in a 15 ml. sterile centrifuge tube. Centrifuge for 3 minutes at 300 G's.

D. Carefully pour off the fluid in the centrifuge tube, leaving the pellet formed from centrifuging in the bottom of the tube. This procedure is known as decanting (pouring off) the supernatant (surface liquid).

E. Pipet 10 ml. PBS with a sterile 10 ml. pipet and pipetting device, and add it to the pelletized cells in the centrifuge tube. Gently agitate and titurate the tube to resuspend the pellet.

F. Again, centrifuge the tube for 3 minutes at 300 G's.

G. Again, decant the supernatant, and resuspend the formed pellet in 1 ml. PBS using a sterile 1 ml. pipet and a pipetting device. This double centrifuging and suspending in PBS serves to wash the cells free of DMEM.

H. Repeat steps B through G using the NS 1 cell line.

I. Transfer the NIE 115 cell suspension to the tube containing the NS 1 cell suspension using a sterile 5 ml. pipet and a pipetting device.

J. Centrifuge the mixed cell suspension 3 minutes at 300 G's.

K. Carefully aspirate (suck up with a sterile 5 ml. pipet and pipetting device) as much of the supernatant as you can, leaving the pellet as dry as possible.

L. **This next step is critical. It is easier to accomplish if two students work together.** Over a styrofoam cup filled with 37° C. water, roll and agitate the centrifuge tube containing the cell pellet. While doing this, with a sterile 1 ml. pipet and a pipetting device, slowly add 1 ml. 50% PEG solution over a 1 minute time period. **Timing and coordination are very important, hence the suggestion for joint effort.**

M. Continue to incubate the tube for one minute over the 37° C. styrofoam cup water bath. Slowly remove the pipet and continue to roll the tube during incubation. **Remember aseptic technique.**

N. Keep gently rolling the tube over the water bath for another minute, while at the same time slowly adding 1 ml. SF DMEM with a sterile 1 ml. pipet and pipetting device.

O. Over a further two to three minute period add 10 ml. additional SF DMEM with a sterile 10 ml pipet and a pipetting device. Continue to gently agitate and roll the cell suspension tube over the water bath.

P. Pelletize this cell suspension by centrifuging for 3 minutes at 300 G's.

Q. Decant the supernatant.

R. Resuspend the pellet in 10 ml. DMEM using a 10 ml. pipet and a pipetting device. Titurate gently with the pipet. Do not remove the pipet from the tube.

S. Using the 10 ml. pipet left in the tube, inoculate 2 Leighton tubes with 1.5 ml. of cell suspension each.

T. Place the remaining cell suspension in a 25 ml. sterile tissue culture flask.

U. Label the new cultures with the proper cell line, passage number, date, and your initials. Make sure that the caps are tight on both flasks and Leighton tubes. Incubate them in a 37° C. incubator for 48 to 72 hours. Do not start hour four until incubation period has elapsed.

Procedure: Hour 4

Students need the following supplies: the incubated cultures in Leighton tubes, centrifuge, 6 each 15 ml. centrifuge tubes, approximately 60 ml. PBS at 37° C., 3 each 5 ml. pipets, 1 each 10 ml. pipet, pipetting device, staining kit, extra 40% methanol, probe, forceps, and 6 microscope slides which have been refrigerated (8 - 10° C.) in 40% methanol.

Note to instructor: the incubated flask from conclusion of hour three should be held in reserve in the event a student group needs to repeat staining procedures of hours four and five. Similarly, incubated flasks from conclusion of hour two also should be held in reserve. Keep flasks in incubator at 37° C., where they can remain viable without fresh media for approximately one week.

Aseptic technique does not need to be strictly observed during this laboratory hour, and thus clean, but not sterile, pipets may be used.

Students will prepare cells for staining during this laboratory hour.

Steps

A. Gently decant the medium from each of the 6 Leighton tubes into separate 15 ml. centrifuge tubes (2 NIE 115 tubes, 2 NS 1 tubes, and 2 potential heterokaryon tubes). Set the centrifuge tubes aside.

B. Gently rinse the Leighton tubes with 5 ml. 37° C. PBS per tube. Use 5 ml. pipets with a pipetting device for this procedure. Care is required so as not to dislodge the cells which should be growing on the sides and coverslip area inside the tubes. Discard the rinsing fluid.

C. Gently remove the flying coverslips from the Leighton tubes using forceps and probe. Carefully immerse each coverslip in the methanol vial from the staining kit. Allow each coverslip to fix for 7 minutes in the methanol.

D. Remove the coverslip and allow it to air dry for 24 hours, or overnight.

When you removed each coverslip you may have seen an opaque area on the coverslip indicating cell growth. If this occurred, hour four procedure is now complete. But when in doubt, or if you suspect that many of the cells did not stick to the coverslip (quite possible with NS 1 cells, and perhaps also the fusion cells), then proceed as follows:

E. Put the appropriate centrifuge tubes prepared in step A into a centrifuge and spin the cells for 3 minutes at 300 G's.

F. Decant the supernatant, and gently titurate to resuspend the formed cell pellet in 10 ml. PBS using a 10 ml. pipet.

G. Centrifuge again for 3 minutes at 300 G's.

H. Decant the supernatant, and resuspend by tituration the formed cell pellet in 5 ml. methanol for 7 minutes. Use a 5 ml. pipet for this procedure.

I. Centrifuge again for 3 minutes at 300 G's

J. Decant the supernatant, and resuspend by tituration the formed cell pellet in 1 ml. methanol. Use a 1 ml. pipet for this procedure.

K. Pipet the cells onto a microscope slide which is propped at a 45 degree angle and which has just been removed from a cold 40% methanol solution. Cover surface of the slide with the solution. Allow the slide to air dry for 24 hours, or overnight.

Procedure: Hour 5

Students need the following supplies: the air dried coverslips and/or microscope slides from hour four of this laboratory, staining kit with fresh Giemsa stain, extra staining materials (Giemsa stain, isopropyl alcohol, distilled water, and xylene), probe, forceps, 6 - 8 microscope slides, permount, toothpicks, and 1 - 3 petri dishes.

During this hour you will cytologically stain and permanently mount both parent and fused cell lines.

Steps

A. Place the air dried coverslips into a Giemsa staining vial, and/or put the air dried microscope slides into petri dishes and cover with Giemsa stain. Allow the cells to fix for three to four minutes in the Giemsa stain.

B. Remove coverslips/slides from Giemsa stain, and dip coverslips three times in the distilled water vial, and/or rinse microscope slides with distilled water.

C. Dehydrate coverslips for 1 minute in the isopropyl alcohol vial, and/or rinse microscope slides with isopropyl alcohol.

D. Allow the coverslips to clear for 1 minute in the xylene vial, and/or rinse microscope slides for 1 minute with xylene.

E. Mount specimens. For flying coverslips, place 3 drops of permount, using a toothpick, on a microscope slide in area where coverslip is to be mounted. Slowly lower the coverslip, **cell side down**, onto the slide. It is best to start at a 45 degree angle with the coverslip so as to trap a minimum number of air bubbles. Carefully press the coverslip to remove bubbles and evenly disperse the permount. For slides, place 4 drops of permount, using toothpicks, over the area of greatest cell number. Lower a clean glass coverslip over the slide. Carefully press to remove air bubbles and evenly disperse the permount.

F. Carefully label the slides and allow them to air dry for 24 hours or overnight.

Procedure: Hour 6

Students need the following supplies: prepared slides and light microscopes.

Students will observe stained cell populations and determine whether fusion between cell lines did indeed occur.

A. Study the pure parent NIE 115 and NS 1 cell slides under the microscope, first on low and then high power.

14. Draw three typical NIE 115 cells in the space provided below. Be very careful to determine the proportion of cytoplasm to nucleus, and note any irregularities in the cell membranes. Label all the cell structures you can identify in your drawings.

15. Describe how the cells physically orient to each other. ______________________________

____

____

____

____

16. Draw three typical NS 1 cells in the space provided below. Be very careful to determine the proportion of cytoplasm to nucleus, and note any irregularities in the cell membranes. Label all cell structures you can identify.

17. Describe how the cells physically orient to each other ____________________

____

____

____

____

18. Are your answers to questions fourteen through seventeen similar to your answers to questions one through seven in laboratory hour one? Why or why not? ____________________

____

____

____

____

B. Study under a microscope the two prepared slides of the (hopefully) fused cells, first under low and then under high power.

19. Can you find any cells which have fused, or look as if they have two nuclei instead of one? ________

20. What proportion of the cells on view in your slide have fused? ____________________

____

Polyethylene glycol does not selectively permit only certain types of cells to fuse. Any cell might have fused with any other nearby cell. Two cells of the same type might have fused. Cells which were not in close proximity with one another when the PEG was introduced would not have fused. You might have created several different heterokaryons.

21. Of the fused cells on your prepared slides, how many of them appear to be fusions of the two parent cell lines? ______

22. Draw as many fused heterokaryons as you can find in your slides in the space provided below. Be careful to determine the proportion of cytoplasm to nucleus, and note any irregularities in the cell membranes. Label all identified cell structures. Also label all those cells which you believe to be heterokaryons fused from both NS 1 and NIE 115 cell lines.

23. One parent cell line is secretory. On many of the cells of this line you can observe small bubble-like structures attached to the cell membrane. These are molecules the cell cytoplasm is secreting. These cells have a relatively large amount of cytoplasm in proportion to the nucleus, because the cytoplasm manufactures the cell secretions. Which parent cell line is this? ______

24. One parent cell line is non-secretory. These cells have a relatively large nucleus in comparison to the cytoplasm. Which parent cell line is this? ______

25. Do the heterokaryons which you believe are the product of the two different cell lines exhibit any of the traits of the parent cell lines? What might these be? ______

Resources

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Terminology

Students should understand the following terms and concepts prior to taking the unit review:

- cell line
- confluence
- decant
- heterokaryon
- myeloma
- neuroblastoma
- supernatant
- titurate

Review
23. Creating a Heterokaryon

Name______________________________

Date______________________________

Matching:

______ 1. polyethylene glycol

______ 2. cell line

______ 3. confluent

______ 4. heterokaryon

______ 5. neuroblastoma

______ 6. myeloma

______ 7. supernatant

______ 8. tituration

______ 9. decant

______ 10. Dulbecco's modified Eagle

A. coming together

B. two nuclei in one cell

C. pour off

D. two cells in one

E. a minimum essential medium

F. excess liquid

G. promotes fusion between cells

H. continuing culture of one cell type

I. cancerous tumor of bone marrow

J. brain tumor

K. promotes cell lysis

L. move back and forth

Essay

1. What do the acronyms DMEM. SF DMEM. PEG. and PBS mean? Discuss their function in cell tissue culture.

2. How might you continue the experiment to produce from your heterokaryons true hybridomas? How would you determine which hybridoma of all those produced by cell fusion is a hybridoma which secretes acetylcholinesterase?

Answers Found: p 269 - #2. 4. 5 & 6: p 270 - #1. 3 & 10: p 276 - #8: p 277 - #7 & 9: Essay #1: laboratory 23: Essay #2: thought.

24. Laboratory

Chicken Embryology

When you have completed this laboratory you should be able to:

1. Describe the changes that take place in a chicken egg during development.
2. List four stages of embryonic development.
3. Diagram an embryo containing the three primary germ layers.
4. Identify the body parts formed from each primary embryonic germ layer.
5. Identify typical structures and embryonic stages during the development of a chicken egg.

Chicken Embryology

Embryology is the study of young animals or plant sporophytes while still contained within a protective structure such as an egg, uterus, or seed. It is one of the most important subjects in all of natural history, as was emphasized earlier in the nineteenth century by Charles Darwin and others. Embryological clues are used to link species and trace ancestries. Since all embryos go through sequenced stages of development, with later structures emerging from primary tissues, similarities during specific stages are used to relate forms which may differ considerably during their adult phases.

The common chicken, used throughout the world as a major protein source, is an easily obtained subject for embryological studies. From hen ovary to finished product, the chicken embryo goes through the typical sequence of fertilization, tissue formation, differentiation, growth, and birth.

In this laboratory, you will learn about the egg laying apparatus of a chicken. You will then take eggs, incubate them, open them at different stages, and study their phases of development. In laboratory twenty six, after some of the eggs have been allowed to hatch, you will study chicken behavior.

Egg Laying

Birds differ from mammals in having only one ovary and oviduct. The ovary of a laying hen contains five to six yellow follicles, which are developing egg yolks with accompanying ova (eggs), and many undeveloped follicles. As a young hen matures, FSH (follicle stimulating hormone) from the pituitary stimulates development of the ovary and its follicles. When the ovary becomes mature enough it secretes estrogen, which causes the development of the oviduct and an increase in blood calcium, fats, and vitamins, all needed for egg formation. Estrogen also causes the pubic bones to spread and the vent (where eggs and feces exit) to enlarge. Progesterone is then secreted by the ovary, and it stimulates the release of LH (leutinizing hormone) from the pituitary, which in turn causes the release of a mature ovum and egg yolk from the ovary into the oviduct. The released yolk is covered by a thin membrane called a vitelline membrane, with the ovum attached to the outside.

The oviduct is also known as the egg canal of a bird, and it has several distinct sections. The first, closest to the ovary, is a funnel-shaped structure called the infundibulum which catches released egg yolks and where fertilization occurs. The egg making process continues whether or not the ovum has been fertilized. Next is the magnum where egg white is deposited around the yolk. The egg's shell membranes are produced in the isthmus. The shell itself is laid around the membranes and their contents in the hen's uterus. The fully formed egg is then laid through the vent. The entire process of forming and laying an egg takes about twenty-seven hours, with the egg remaining longest in the uterus.

The Yolk

The yolk provides necessary nutrients for the developing embryo. Ninety percent fertility is considered good fertility in chicken eggs. Look for a small bullseye dot of yolk-free cytoplasm attached to the egg yolk membrane. That is the egg (ovum) itelf. Chicken sperm can survive in an egg canal for one month. Therefore, one mating will provide a hen with sufficient sperm for up to one month of fertile egg laying. If it has been fertilized, the bullseye will have a tiny lump in its center called a blastodisc. Cleavage and development occur only within this germinal disc-shaped area.

Chicken embryos develop as the egg travels down the egg canal. When a fertilized egg is laid, the blastodisc will be multicellular. Development stops when the egg is laid, and it does not start again until the temperature is brought back up to a chicken's body temperature of 38°C. The most critical days in terms of survival for a chicken embryo are on the third day of incubation and just before hatching.

One way to determine the age of a chicken egg is to crack it into a small bowl, and observe the roundness of the egg yolk. If the yolk stands well above the egg white in a semi-round shape, the egg is fresh. The flatter the yolk, the older the egg.

Sometimes an ovum and yolk produced by the ovary misses the infundibulum and gets lost in the abdominal cavity. If this happens to a hen, she will appear to be in a laying condition but does not lay a usable egg. Chicken farmers call such hens internal layers.

The Egg White

Once the yolk has been caught by the infundibulum, it passes to the magnum of the oviduct, where the albumen, or egg white, is deposited around the yolk. The egg white, like the yolk, is a complex protein. Albumen serves as a physical protection, or shock absorber, for the developing embryo. Albumen contains antimicrobial substances which protect the embryo from bacterial infection. Albumen also provides the chick with a warehouse of amino acids to draw on for protein synthesis, and the liquid is the chick's only source of water.

As the egg travels down the egg canal, it twists like a corkscrew. This mechanically is the easiest method for squeezing through tight spaces. Often the albumen becomes tangled at the front and rear ends of an egg through this twisting motion. This tangled albumen is called chalaza; you can see these whitened rope-like structures in most bird eggs. Chalza has no function in an egg. (Blood spots also have no function, except for demonstrating that chickens too can suffer from hemorrhoids).

Membranes

Two shell membranes are added to the egg in the isthmus section of the oviduct. The thick outer membrane and the thin inner one stick together, except at the large end of the egg, where they separate to form a dead air space. This air space is very small when an egg is first laid, but as an egg cools the space expands. One way to tell if an egg is old is by the size of its air space (the larger, the older). This air space is very important to the developing chick. One to three days before hatching the chick becomes an air breather when it punctures the inner membrane and sticks its head into the air space. When this happens you can hear the chick cheeping inside the egg. It must hatch before the air in the air space is expended.

The Shell

In the uterus the eggshell, formed almost completely of calcium carbonate, is deposited. The shell attaches to the outer shell membrane. The hard shell protects the embryo and its food supply, but it is sufficiently porous to allow gas exchange with the environment. Eggshell color is determined by the deposition of pigments in the calcium carbonate as the shell is being deposited. Pigmentation is genetically determined by chicken variety. White eggs simply have no pigmentation. Some chickens called Araucanas lay Easter eggs, or eggs whose shells are pigmented in different pastel colors.

Laying hens require large amounts of calcium in their diet. If they do not obtain enough calcium, problems may develop in the hen house. Eggs will have thin shells. Thin shells lead to broken eggs. Once one chicken in a flock discovers that eggs are good to eat, they all can learn to crack and eat eggs, whether or not they are thin shelled. The only way to stop such cannibalism is to kill all members of the flock.

Light and Egg Production

Light is extremely important to egg production, even though a rooster is not. When young hens reach a certain stage of sexual maturity, light stimulates the egg laying process. This process continues whether or not the hen has been fertilized. The approach of winter with its shortening days reduces egg laying. This is why many chicken farmers provide artificial light in their hen houses during the short day months. Young hens will continue laying as long as a fourteen to sixteen hour day is maintained. Also, if laying begins at too early an age, the eggs will be small. If laying is delayed, by keeping the hens in an artificially shortened-day environment, eggs will be larger when production is stimulated.

Optional Activity

Although not detailed in the following procedures, some classes may want to obtain a freshly killed laying hen for dissection. The instructor and/or students may then demonstrate the egg forming processes within the hen. Several eggs in different stages of development should be seen within the dissected oviduct.

Pre-lab

Supplies neeed:

Equipment

microscope
dissecting scope
finger bowl
teasing needle
probe
dissecting scissors
plastic spoons
waste container
38°C. incubator
large beaker
thermometer
watch glass
heat source

Materials

unfertilized egg
4 fertilized eggs
0.9% NaCl solution
filter paper

Special Preparations

1) Stabilize the incubator's temperature at 38°C., and maintain that temperature constant for at least twenty four hours before placing eggs in the chamber. Make sure the incubator maintains the high humidity eggs need for proper development.

2) You can simultaneously hatch birds which incubate at the same temperature as chickens. For example, pheasant and quail eggs hatch in 23 days, duck eggs hatch in 28-33 days (depending on species), guinea and turkey hatch in 27 days, and geese hatch in 28-33 days.

3) The 0.9% saline solution is to be heated and used at 38°C. The instructor may prepare and heat the approximately two liters required for an entire class. Alternatively, each student group may prepare the heated saline solution which will be used to lessen the shock of embryonic transplant from the egg. Using warmed saline solution increases the time students may observe a living embryo.

4) The unfertilized eggs specified for hour one may be purchased from any grocery store more cheaply than fertilized eggs. Mass egg production techniques are such that commercial eggs usually are unfertilized. Of course there are exceptions, and lab participants may wish to verify infertility as they proceed with hour one. Hour one procedures may continue with a fertilized egg.

5) This laboratory, with proper planning, provides the chicks to be imprinted during laboratory twenty-six. By obtaining and continuing to incubate more fertile eggs than are needed for this experiment, students will be able to duplicate Lorenz' classic experiment on fowl imprinting. When calculating the number of fertile eggs to be incubated, the instructor should allow for approximately twenty percent sterility and death, in addition to the fertile eggs or chicks required for each group during laboratories twenty-four and twenty-six.

Time Required

This chicken embryology laboratory requires four classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the following supplies: unfertilized egg, 10-15 ml. 0.9% NaCl solution, dissecting scope, finger bowl, teasing needle, probe, watch glass, egg waste container, dissecting scissors, filter paper, and plastic spoon.

You will study the structure of an unfertilized egg during this laboratory hour.

Steps

A. Carefully carry an egg, same side up as it is found, from the egg carton to your work area. While holding the egg same side up, place a small crack in the top of the shell with the blunt end of the probe or dissecting scissors. Try not to puncture or damage the shell membrane. The shell membrane toughens as an embryo develops, and leaving it intact will be easier with fertilized eggs. Keeping the egg as steady as possible, carefully chip away the top third of the shell, leaving an opening through which the entire top of the egg yolk can be seen. Study the egg's structure.

1. Identify and label the parts of a chicken egg indicated by the lines in figure one below, writing on or next to the lines.

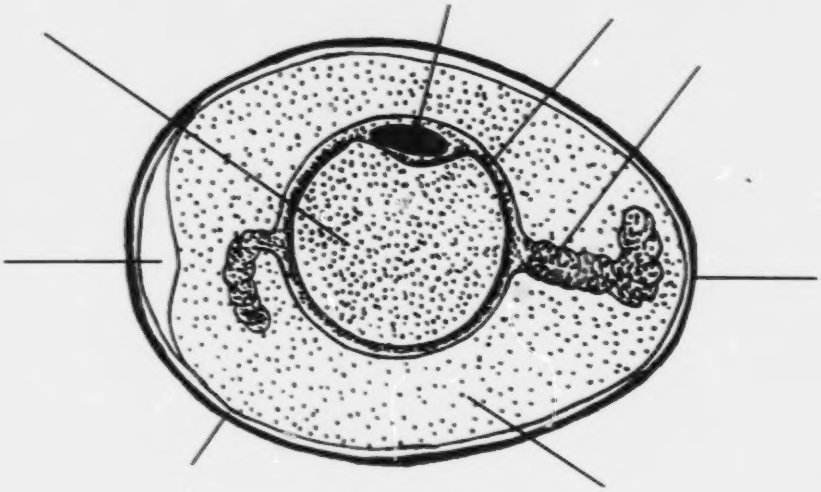


Figure 1

B. The bullseye, or germinal disc, should be floating on the upper side of the yolk, depending on the end upon which the egg has been resting. Therefore, you should see the germinal disc on the upper surface of the yolk. Rest the egg in the finger bowl while studying the germinal disc with the aid of a dissecting scope.

If the germinal disc is not on the upper side of the yolk, carefully pour out the contents of the egg, without breaking the vitelline membrane, into the finger bowl. Search for the germinal disc. When found, turn the yolk so that the disc is on its upper surface.

C. Cut out a paper wand from filter paper using dissecting scissors. The wand itself should have a large enough hole so that the germinal disc of the egg can fit inside. It should look something like figure two when completed (imagine a soap bubble blowing device with handle and loop).

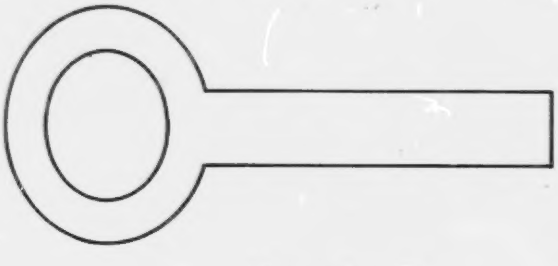


Figure 2

D. Add a little saline solution to a watch glass (if you were dissecting a living embryo, and not an infertile egg, the saline solution would need to be 38°C.). Lay the circle of the filter paper wand on the yolk so that the germinal disc shows through the hole. Press the filter paper gently so that it becomes wet all over and sticks to the vitelline membrane.

E. Carefully cut with dissecting scissors the vitelline membrane in a circle around the outside of the wand, to free the germinal disc from the yolk.

F. Lift the wand slowly so that the embryo lifts from the surface of the yolk. Lay the disc in the saline solution in the watch glass, and study it through the dissecting scope.

2. Draw the structure of the germinal disc in the space provided below, noting if you have opened a fertilized or unfertilized egg.

Procedure: Hour 2

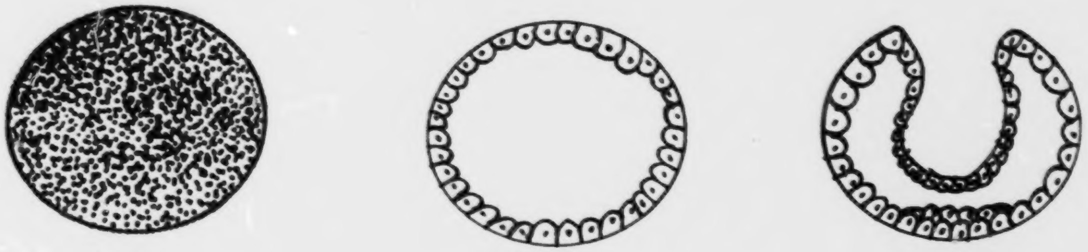
Students need the following supplies: fertile egg incubated for two days, microscope, dissecting scope, finger bowl, egg waste container, teasing needle, probe, watch glass, dissecting scissors, 0.9% saline solution, heat source, and beaker.

During this laboratory hour you will dissect a fertile chicken egg incubated for two days.

Early Embryological Development

The first cell of a new organism is the zygote, made from the fusion of sperm and egg. The zygote mitotically divides, and divides again, and these divisions in multicellular animals stick together to form a many celled blastula, or hollow ball of cells. The cavity of the blastula is called a blastocoel, and when the ball's surface no longer supports the dividing cells, it suddenly caves in to form the gastrula, which looks like a deflated basketball. This collapsing process is termed gastrulation, and the new cavity caused by gastrulation forms the primitive gut, called the archenteron, of the embryo. The blastopore is the lip of the collapsed blastula. Because of gastrulation, there are now two primary germ layers in the embryo, an inner one and an outer one. These are called endoderm and ectoderm, respectively. Primitive organisms with only two cell layers stop development at this early stage; most animals continue to form a third cell layer called the mesoderm.

The three early embryological states — zygote, blastula, and gastrula — may be summarized as shown in figure three.



Zygote **Blastula** **Gastrula**

Figure 3

Initially the embryo does not become larger, but instead becomes denser and more complicated. When sufficient cellular division has occurred, cells begin migrating from the blastopore into the space between the endoderm and the ectoderm, eventually forming the third layer, the mesoderm. This signals the start of the triploblastic embryo stage. During the triploblastic embryo stage organ formation begins. This is known as organogenesis.

In general terms, the skin, nervous system, sensory organs, and the lining of the mouth and anus form from the ectoderm. The endoderm gives rise to the inner lining of the digestive tract and the formation of organs related to it. The endoderm and the ectoderm jointly (in the area of the primitive blastopore) give rise to the mouth and anus. The mesoderm gives rise to all other tissues, organs, and systems.

Organogenesis is accomplished by several cellular growth activities, such as invaginations (inpocketings), evaginations (outpocketings), cell thickenings, and epiboly (covering one cell layer with another).

Embryonic Induction

It was long theorized that there must be something which controls and induces tissue differentiation and morphogenesis. The German graduate student Hilde Mangold demonstrated in 1924 that the lip of the blastopore acts as an organizer. She cut the dorsal lip from the embryo of one salamander and grafted it into the belly region of another embryo. When the embryo that had received the graft developed, it was found that a second embryo had formed within its tissues, a sort of Siamese twin to the first. Because the two salamanders differed in color, it was possible to distinguish the tissues of the host embryo from those of the transplant. The grafted lip developed into a notochord, just as it would have done in its original embryo, but most of the second embryo was composed of tissues of the host, as could be seen from color differences. This experiment has been repeated many times, always with the same result. The dorsal lip of the blastopore consistently demonstrates an ability to organize tissues.

The process of organizing other cells into differentiated tissues is known as embryonic induction. It is now known that there are many tissue-forming inducers which act during the development of an organism. Induction occurs if there are certain chemical exchanges between cells. Induction and differentiation do not occur if these chemical exchanges are blocked. Studies aimed at isolating all the chemicals and explaining the triggering processes are now an active area of embryological research.

The chemical exchanges can be of a very general nature, in that the capacity of the organizer to induce differentiation is not necessarily species-specific. For example, secondary neural plate formation can be induced in a chicken embryo by grafting tissue from a rabbit embryo.

The French Flag Theory

Researchers have theorized that inducers might operate on a cell per its position within a group. This "French flag" theory states that if there is a group of undifferentiated cells, a chemical gradient is established through diffusion and that organogenesis is determined by differing concentrations of the chemical within the group of cells. While the cells are still undifferentiated, one cell can be removed, and the cells will adjust their roles automatically. If two cells simultaneously assume the lead position, development will include a degree of Siamese twinning, in that there will be double cellular differentiation of the cells positioned immediately behind this doublet.

Yolk Types in Embryonic Development

A chicken egg represents only one of four basic types of egg development. The way an egg divides, or undergoes cleavage, is an important aspect of its growth. A chicken egg represents discoidal cleavage, in that the yolk is so massive that it changes the shape of the embryo to that of a flattened disc. Discoidal cleavage is typical of bony fishes, reptiles, birds, and monotremes. Because of the way the blastula is flattened, the blastulas of these egg laying animals are called blastodiscs. The difference between a sea urchin blastula and a chicken blastula is illustrated in figure four on the following page.

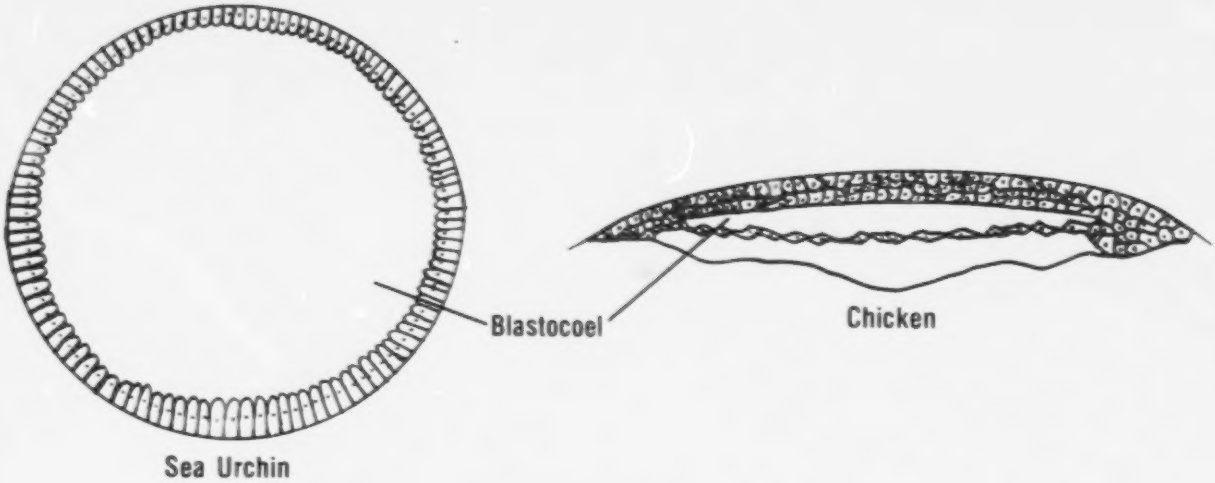


Figure 4

Another type of egg is one in which the yolk is scattered uniformly in the cytoplasm of the embryo and both yolk and cytoplasm develop equally. This type undergoes equal cleavage of cells. Placental mammals and echinoderms both undergo this type of embryological development.

There is a third type of cell which demonstrates unequal cleavage. This type of egg has a vegetal pole (yolk), which divides slowly, and an animal (embryo) pole, which divides much faster.

The fourth type, superficial cleavage, is typical of arthropods. The yolk forms around a nuclear zygote. When division of the nucleus occurs, the divided cells migrate through the yolk to the outer periphery of the egg. The embryo then develops on the outer periphery. Changing egg orientation, and thus changing gravity during development, can kill insect embryos.

The Blastodisc

When a fertile chicken egg is laid, the blastodisc already will have become a white mass about two millimeters in diameter attached to the yolk. The cells will number approximately 100,000 and group in two layers; the top layer will give rise to the ectoderm and the lower layer to the endoderm. If incubation is begun, a line known as the primitive streak (technically the blastopore) will appear shortly. Cells migrating through the primitive streak form the mesoderm and part of the endoderm. When gastrulation is complete, mesodermal tissue near the center of the embryo pinches off to form the notochord and the pairs of somites, or primitive segments, which lie in rows along the notochord. The notochord induces the ectoderm above it to form the neural plate, and subsequently the neural plate folds to form the neural tube. The neural tube differentiates into the brain and spinal cord.

Extraembryonic Membranes

Extraembryonic membranes connect the developing embryo to its food supply, the yolk. During embryological development the extraembryonic membranes are more obvious than the embryo itself. Extraembryonic membranes form as extensions of the blastodisc. Each membrane is formed from a combination of two of the three basic tissue types, endoderm, mesoderm, or ectoderm.

The yolk gradually is enveloped by the yolk sac membrane. Blood vessels in the yolk sac membrane carry food from the yolk to the embryo. An amniotic sac encloses the embryo; it is filled with amniotic fluid which protects and cushions the embryo. The amniotic sac is composed of two membranes, an inner amnion and an outer chorion. The allantois, a pouch attached to the embryo inside the amniotic sac during early stages of development, functions as a garbage can for embryonic wastes. This membrane can be found stuck to the shell after the chick hatches. The allantois, in the later stages of embryonic development, becomes the chorioallantoic membrane, formed by the fusion of the allantoic wall with the chorion. It acts as a respiratory membrane for the embryo and is plentifully supplied with blood vessels.

During this laboratory hour you will study a two day old chick embryo. A two day old chick embryo mounted on a slide should appear similar to the drawing in figure five below. If conditions for incubation have been less than ideal, the embryo might still be living but not be as advanced in appearance as the drawing suggests.

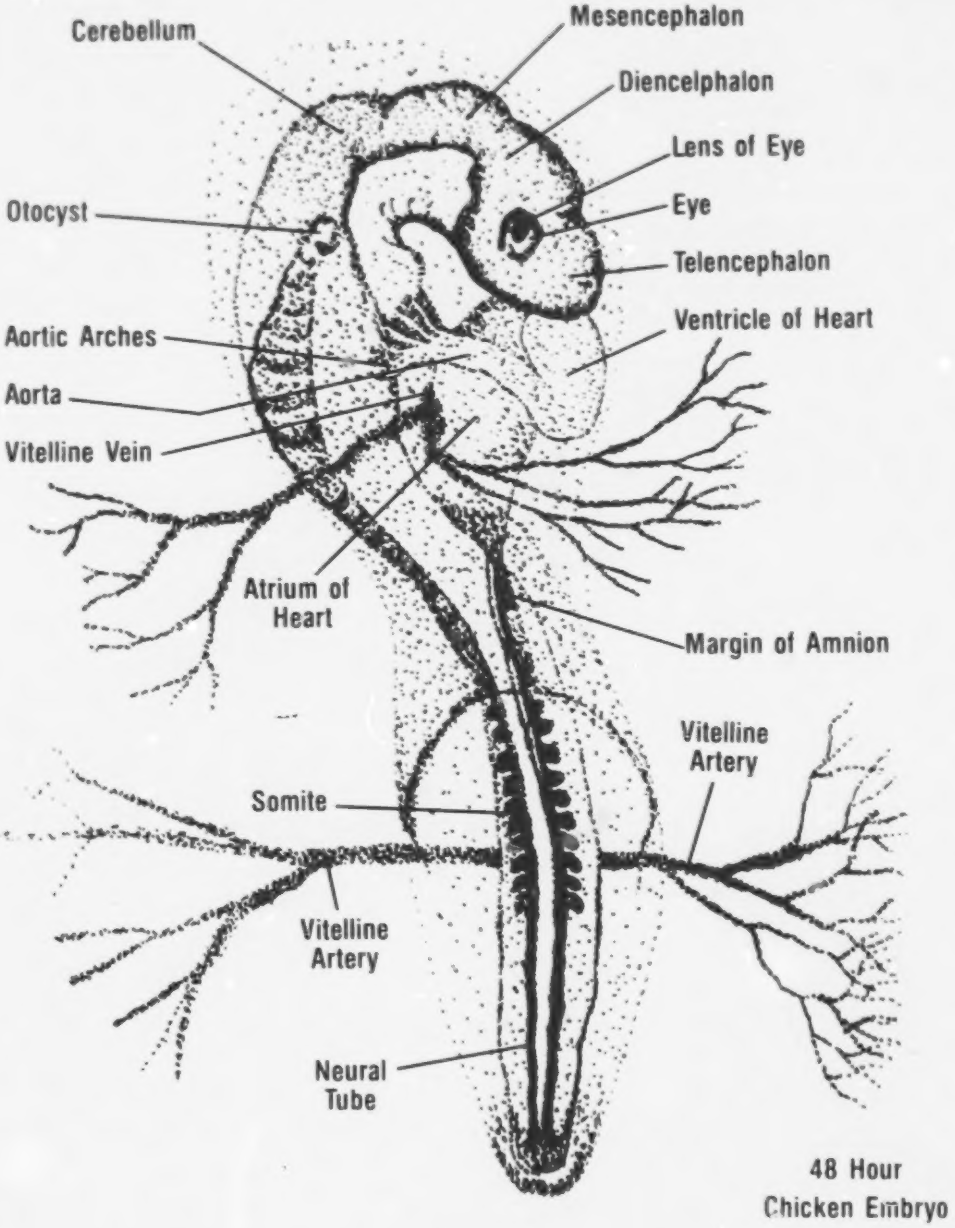

Cerebellum
Mesencephalon
Diencelphalon
Lens of Eye
Otocyst
Eye
Telencephalon
Aortic Arches
Ventricle of Heart
Aorta
Vitelline Vein
Atrium of Heart
Margin of Amnion
Vitelline Artery
Somite
Vitelline Artery
Neural Tube
48 Hour
Chicken Embryo

Figure 5

Steps

A. Use instructions followed during hour one of this laboratory to open and study a two day incubated chicken egg. Note the exact time you expose the embryo from the surrounding albumen. Use the dissecting scope to clarify embryonic structures.

Note that a two day old chick embryo has started to twist to the right so that the head region is now lying on the chick's left side. This is the process of torsion, which will continue until the whole embryo is lying on its left side. At the same time, the embryo curls up so that the neural tube takes the shape of an upside down backwards question mark, with a bend in the area of the hindbrain.

B. Locate the extraembryonic membranes and all visible embryonic structures.

3. Compare your embryo with figure five on the previous page. Describe any differences.

4. Where is the heart, and is it beating?

Note: if the embryo is dead, obtain another egg from the instructor and repeat steps A and B.

5. Count and record heart beats per minute.

6. How long does the heart continue to beat in your embryo once it is exposed?

7. Blood circulation usually begins when the embryo's somites number sixteen. How many somites are visible in your specimen?

8. Is blood circulating in the extraembryonic membranes?

9. Trace the flow of blood from the ventricle of the heart through the aorta and then through the aortic arches in the region of the pharnyx. Diagram the blood flow structure and direction of flow in the space provided below.

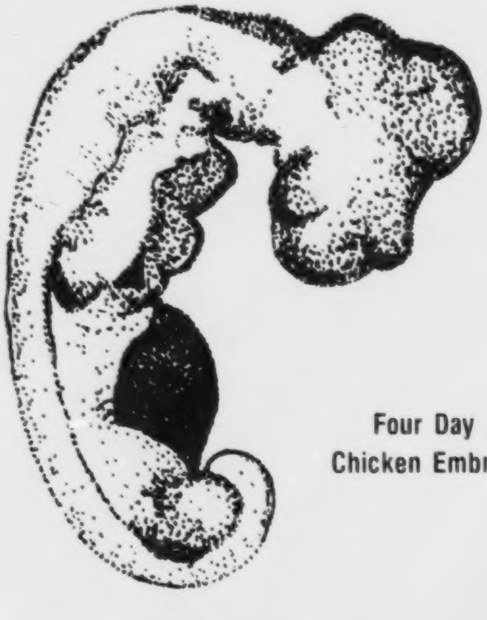
C. Using a filter paper wand and 0.9% warmed saline solution in the bottom of a watch glass, carefully transfer the embryo to the saline solution. (This technique was outlined during hour one of this laboratory).

10. Locate the parts of the brain. The optic cup has induced formation of the lens of the eye by the ectoderm, and the neural tube has induced formation of the primitive ear. Diagram parts of the brain and sensory organs in the space provided below.

Procedure: Hour 3

Students need the following materials and supplies: fertile egg incubated four days, microscope, dissecting scope, finger bowl, egg waste container, teasing needle, probe, watch glass, dissecting scissors, 0.9% saline solution, heat source, and beaker.

During this laboratory hour you will study a four day old chicken embryo. A four day old chick embryo mounted on a slide should appear similar to the drawing in figure six below.



Four Day
Chicken Embryo

Figure 6

Steps

A. Open and study a four day incubated chicken egg, using the procedures used during hours one and two. Note the exact time you expose the embryo from the surrounding albumen. Use the dissecting scope to clarify embryonic structures.

B. Locate the extraembryonic membranes and all visible embryonic structures.

11. Compare your embryo with figure six above. Describe any differences:

Your embryo should have developed about forty-one somites, and the yolk should be partially covered with prominent blood vessels. The brain should be divided into three distinct regions, with the eye easier to see because tissues have beome thicker in that area.

C. Remove the membranes surrounding the embryo so that you can see the heart and limb buds.

Note: If the embryo is dead, obtain another egg from the instructor and repeat steps A-C above.

12. Record heart beats per minute:

13. Note how long the heart continues to beat in your embryo once it is exposed?

14. Describe how blood is circulating in the extraembryonic membranes of your specimen?

D. Use a probe or teasing needle to straighten out the embryo so that the pharyngeal clefts and pouches are visible. (These structures appear at some stage in all vertebrate embryos).

15. What membrane completely encloses the embryo?

The fluid-filled sac at the posterior end of the embryo is the allantois. It will expand and eventually fuse with the chorion and become highly vascularized.

16. What are the two functions of the allantois?

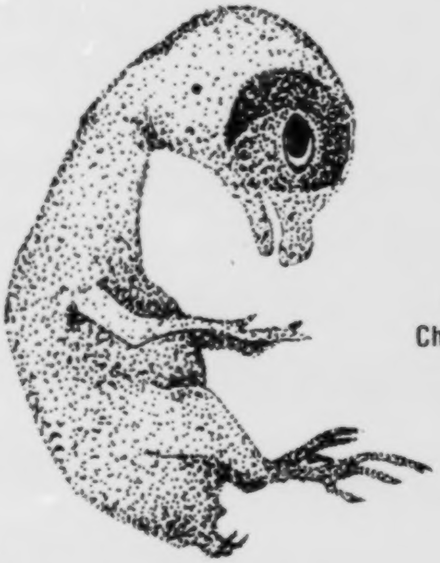
E. Using a filter-paper wand and 0.9% warmed saline solution in the bottom of a watch glass, carefully transfer the embryo to the saline solution.

17 Diagram the embryo in the space provided below. Label all visible structures.

Procedure: Hour 4

Students need the following materials and supplies: fertilized egg incubated ten to twelve days, dissecting scope, finger bowl, teasing needle, probe, watch glass, egg waste container, and dissecting scissors.

During this laboratory hour you will study a ten to twelve day old chicken embryo. This will be the last view of a chicken embryo before hatching. All major developmental processes occur during the first six days of development. The embryo then grows larger and matures during the rest of the incubation period, until it hatches at twenty-one days. Figure seven below represents a generalized view of a ten to twelve day old embryo.



Eleven Day Chicken Embryo

Figure 7

Steps

A. Open a chicken egg incubated ten to twelve days. Note the exact time you expose the embryo from the surrounding albumen. Use the dissecting scope to clarify embryonic structures.

18. Compare your embryo with figure seven on the previous page. Describe any differences: ____________

The embryo should have developed large, prominent, pigmented eyes by day eight of incubation. The iris of the eye should be clearly visible, with eyelids developing to cover the eyes. The small opening behind the eye is the ear. At eleven days the limbs are developing, and toes should be very distinct, with claws on the ends of the toes. There should be wing "fingers" on the wing bud, even though the chicken does not have fingers in later life. The chicken's beak should be well developed. The brain is visible through the transparent skull, and the skin should be covered by prominent feather buds. Note how large the head is in relation to the trunk.

19. Count and record heartbeats per minute: ____________

Note: if the embryo is dead, skip questions twenty and twenty-two through twenty-four.

20. How does the rate of this embryo's heartbeat compare with the heartbeat of a four day old embryo?

21. Do you think that the heartbeat of a newly hatched chick will be faster or slower than that of a ten to twelve day old embryo? Why? ____________

22. Record how long the heart continues to beat once the embryo is exposed: ____________

23. Was blood circulating in the extraembryonic membranes of your specimen while still alive? ____________

24. Describe any attempts by the embryo to move: ____________

25. Note the size of the attached yolk. How does it compare with the yolk of an unfertilized egg? Why has it changed? ____________

26. Compare the relative size of head and trunk of this embryo, and those examined earlier, with that of a hatched chick.

27. How big is the eye relative to the size of the head? How does this proportion, eye to head, compare with both younger embryos and newly hatched chicks?

28. Are the wings as big as the legs in your specimen? Why is growth (not) uniform in the two appendages?

29. Would you conclude that all parts of an organism grow at the same rate throughout life? Why or why not?

30. Note where the head of the embryo is in relation to the dead air space of the egg. Draw the embryo in the space provided below, labeling all embryonic and extraembryonic structures visible in your specimen.

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Terminology

Students should understand the following terms and concepts prior to taking the unit review:

- albumin
- allantois
- amnion
- blastodisc
- blastopore
- blastula
- chalaza
- chorion
- cleavage
- dead air space
- egg canal
- embryology
- extraembryonic
- follicle
- French flag theory
- gastrula
- infundibulum
- isthmus
- magnum
- primitive streak
- triploblastic embryo
- vent
- vitelline membrane
- yolk sac membrane
- zygote

Review

24. Chicken Embryology

Name__________________________________

Date___________________________________

Identification: match letters pointing to parts of chicken egg in illustration to numbered labels listed along left margin.

______ 1. yolk

______ 2. shell membrane

______ 3. vitelline membrane

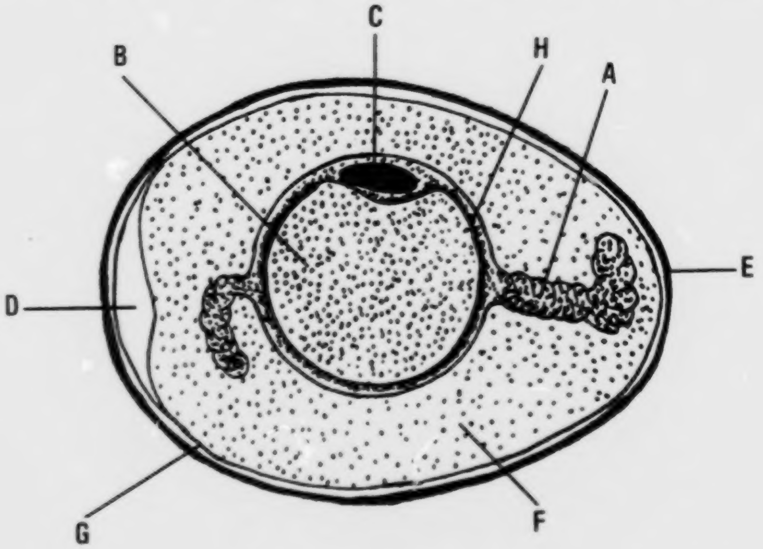
______ 4. chalaza

______ 5. blastodisc

______ 6. shell

______ 7. air space

______ 8. albumen



Multiple choice

______ 9. In a chicken egg, cleavage begins in the
  a. egg after the shell is formed
  b. in the oviduct
  c. in the uterus
  d. in the egg when the chicken sets on it

______ 10. When sperm and egg fuse, the resulting one cell is called a
  a. blastula
  b. mesoderm
  c. zygote
  d. germ layer

______ 11. The primitive gut of an embryo caused by gastrulation is called the
  a. endoderm
  b. blastocoel
  c. blastopore
  d. archenteron

______ 12. Outpocketings in embryology are called
  a. evaginations
  b. invaginations
  c. thickenings
  d. differentiations

______ 13. The endodermis forms tissues of the
  a. mouth
  b. stomach
  c. heart
  d. skin

______ 14. Developmentally, all the major structures of a chicken embryo are formed by day
a. two
b. six
c. ten
d. eight

______ 15. The organ to first reach a functional state in an embryo is the
a. stomach
b. heart
c. liver
d. lungs

______ 16. The lead cell in the French flag theory, which determines cellular differentiation in those cells following it, can be defined as a/an
a. repressed cell
b. competent cell
c. organizer
d. Down's syndrome cell

______ 17. Somites differentiate into
a. vertebrae
b. brain
c. intestine
d. ribs

______ 18. These chickens lay Easter eggs.
a. Rhode Island Reds
b. Araucanas
c. Plymouth Rocks
d. Barred

______ 19. The infundibulum is part of the
a. gastrula
b. uterus
c. blastula
d. oviduct

______ 20. This hormone causes the release of a mature ovum and egg yolk from the ovary of a chicken into the oviduct.
a. FSH
b. estrogen
c. LH
d. progesterone

Answers Found: p 285 - #20; p 286 - #19; p 287 - #18; p 289 - #1, 2, 3, 4, 5, 6, 7, & 8; p 290 - #10 & 11; p 291 - #12, 13 & 16; p 292 - #9 & 17; p 294 - #15; p 297 - #14.

25. Laboratory
Cat Dissection

When you have completed this laboratory you should be able to:

1. Locate and describe the action of at least twenty cat muscles.
2. Trace the digestive path of a cat from mouth to anus.
3. Trace the circulatory and respiratory systems of a cat.
4. Identify the excretory and reproductive structures of a cat.
5. Identify the basic structures of a cat's nervous system.
6. Identify as to type and function at least fifteen bones and three joints in a cat.

Introduction

The cat is popular for mammalian anatomy studies. Its size is convenient for laboratory dissection, and it represents basic anatomical features of the class *Mammalia*. Students who continue on to a medical career will dissect and study human cadavers in great detail. This laboratory introduces requisite anatomical knowledge and dissection skills.

Pre-lab

Supplies needed:

Equipment

dissecting pan
large plastic bag with tie
bone scissors
microscope
razor
colored pencils
dissecting scissors
probe
forceps
slides
coverslips
ruler

Materials

preserved cat
straw
optional: disposable gloves

Special Preparations

1) Purchase double latex injected cats; such specimens are preferable for study of the circulatory system.

2) If students are squeamish, the instructor may provide disposable latex surgical gloves, which are available in boxes of one hundred. However, students will gain more from the dissection if bare hands are used.

3) Standard dissecting pans are too short for cat dissections. Earth science stream tables, used in the study of soil erosion, are a good size for cat dissections.

Time Required

This cat dissection laboratory requires eight classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the following supplies: cat, dissecting pan, probe, forceps, dissecting scissors, colored pencils, and large plastic bag and tie.

You will study the external structure of a cat during this laboratory hour and begin skinning the specimen.

Steps

A. Study the external structures of the cat. Locate the ventral and dorsal surfaces of the specimen. The cat's trunk is divided into the chest, or thorax, and a belly, or abdomen. Note the two external characteristics unique to all mammals: hair and mammary glands.

1. Describe your particular cat so that it can be distinguished from other specimens (fur color, etc.).

____

____

2. How many nipples, or teats, does your cat have? ____________

Most mammals have separate urogenital and anal openings. The anal opening of a cat is located at the base of the tail, dorsal to the urogenital opening. In males, the urogenital structures consist of a penis and the testes, contained in a sac called the scrotum.

3. Is your cat male or female? ____________

Study the cat's feet. In a cat the claws are retractable, and the epidermis on the bottom of the cat's feet is thickened into pads.

4. How many pads are on each foot? ____________

5. Do cats walk on the entire soles of their feet, on their toes, or only on their claws? ____________

6. How can you tell from observing a dead cat how it walked? ______________________________

____

____

____

Study the cat's head. Locate the face. The lips around the mouth are well developed, and the upper lip is cleft in the center by a groove called the philtrum. The nares are on a naked nose. The eyes have upper and lower lids as well as a small nictitating membrane.

7. In the space provided below draw a cat's eye, with its surrounding structures. Label the lids and membranes.

8. Notice that the ears possess a long flexible external fold. This fold is called the pinna, and it directs sound waves into the external auditory meatus of the ear. In the space provided below, draw a cat's ear, and label the external structures.

B. Open the cat's mouth. Note the placement of the tongue. Study the placement of both upper and lower teeth. In the front of the mouth, there are six similar-appearing teeth on both the upper and lower jaws. These are incisors. On each side of the incisors there is a canine tooth, for a total of four canine teeth. Canine teeth are long and pointed. After each canine tooth there are two premolars, for a total of eight premolars. An adult cat will have a molar tooth after the premolars on each side of the jaws, for an additional four teeth. Juvenile cats will not have molar teeth.

9. In the space provided below, diagram your cat's upper and lower jaws. In the diagram place the cat's teeth as you observe them. Label the teeth as to type.

10. Is your cat specimen an adult or a juvenile? How can you determine this? ______________________

____

____

C. You will now skin your specimen. Use dissecting scissors, preferably those with one blunt tip. Insert the tip between the skin and muscles of the cat in the neck opening made during embalming of your specimen. Do not cut into the musculature of the animal. Continue the cut to the front of the neck, then up to the chin and down the body as illustrated in figure one.

If your specimen is a female, cut around each mammary gland, and leave as much teat as possible with the gland.

Cut around the mouth, ears, eyes, wrists and ankles, leaving the fur on the feet, chin, ears, and eye areas, and carefully separate the skin from underlying tissues with the blunt end of your scissors or probe.

Note: Students will begin the skinning process during the first laboratory hour, but they will need to complete it during the second laboratory hour. When class time has expired, store the cat in a large plastic bag on the dissecting tray, along with the dissecting utensils. Students should label their specimens.

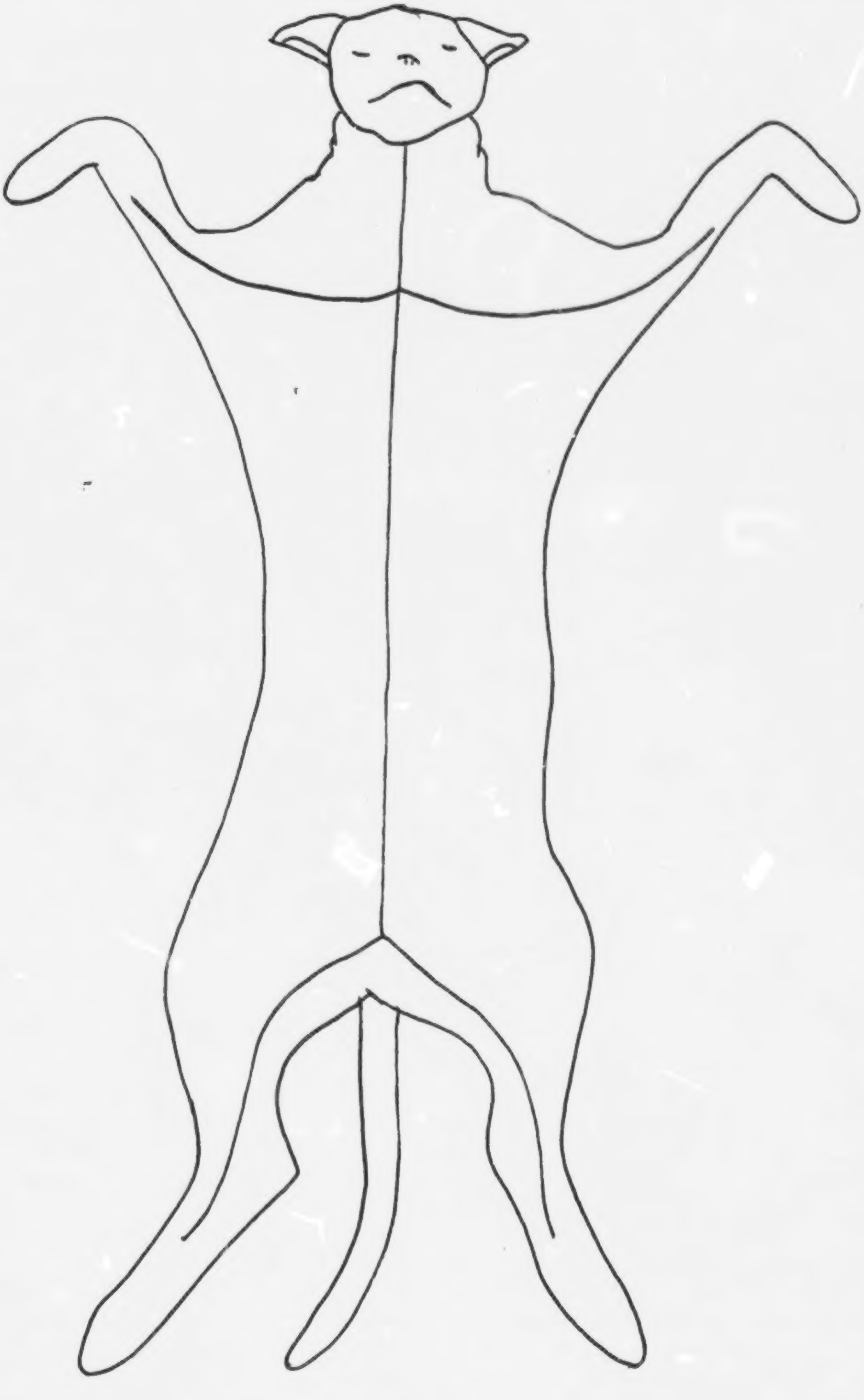


Figure 1

Procedure: Hour 2

Students need the following supplies: cat, dissecting pan, probe, forceps, dissecting scissors, and large plastic bag and tie.

During this laboratory hour you will complete the skinning process and then study the musculature of a cat.

Steps

A. Complete the skinning process begun in hour one of this laboratory.

B. Study the muscles exposed by the skinning process. All striated muscles are covered with a tough connective tissue known as fascia. Fascia near the skin is spongy and is known as superficial fascia. This is what you separated when skinning the cat. A second inner layer, deep fascia, is rather thin and somewhat iridescent in appearance.

Observe the calf of a hind leg with its musculature. The leg calf is the gastrocnemius muscle. The muscle has two ends, known as heads. Skeletal muscles are connected by tissue bands to the bone that they move. The point where a muscle connects to a bone that it moves is known as the insertion of the muscle. The proximal attachment of the muscle (at the other end) is known as the origin. The origin attachment usually is to another bone, but one which that particular muscle does not move. The function of the muscle is to move the animal's bone in a specific way. This is known as the muscle's action.

Look for individually covered bundles of muscle fibers within the muscle. The thick part of a muscle is known as the belly, and the cords of connective tissue at the ends of the muscle are called tendons. If the cords were flat, as they are on the animal's trunk, they would be called aponeuroses.

Aponeuroses attach skin muscles which lack skeletal attachments. Observe the side of the cat's body. You should see a large sheet of skin muscle called the cutaneous maximus. The cutaneous maximus originates on each side of the fascia of the latissimus dorsi and of the ventral pectoral muscles. See figure two to locate these structures.

Study figure two, on the following page, which diagrams a lateral view of superficial trunk and limb musculature. Find on your specimen the muscles labeled in the diagram.

Study figure three, on page 310, which diagrams a lateral view of deep trunk and limb musculature. Find on your specimen the muscles labeled in the diagram.

Study figure four, on page 311, which diagrams a ventral view of superficial and deep trunk and limb musculature. Find on your specimen the muscles labeled in the diagram.

11. Determine the location and describe the action of the muscles listed in figure five on page 312.

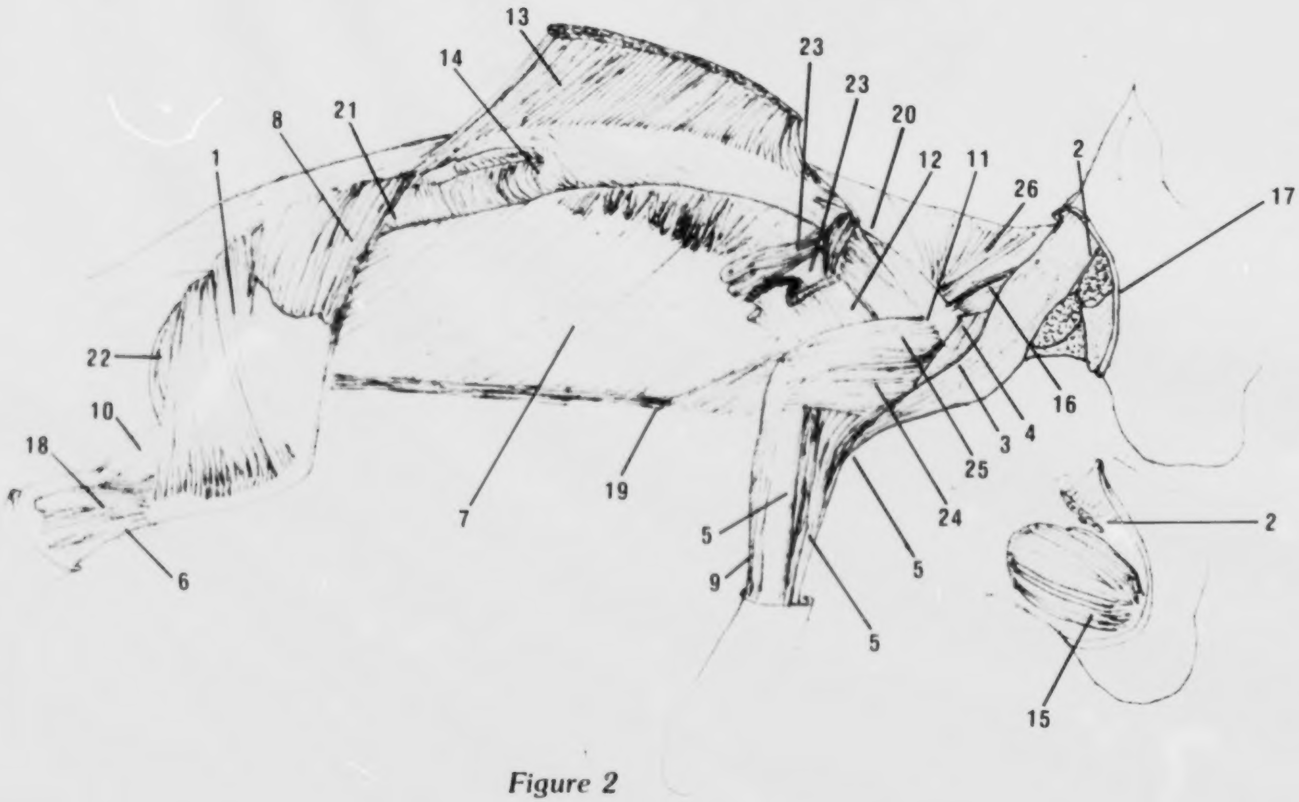


Figure 2

1. Biceps femoris
2. Cleidocephalicus
3. Deltoidus - pars acromialis
4. Deltoidus - pars scapularius
5. Extensors
6. Extensor digitorum longus & Tibialis cranialis
7. External oblique
8. Fascia over Gluteus medius & Gluteus maximus
9. Flexor digitorum profundus
10. Gastocnemius
11. Infraspinatus
12. Latissimus dorsi (insertion cut)
13. Latissimus dorsi (origin cut)
14. Longissimus
15. Masseter
16. Omotransversarius
17. Parotid gland
18. Peroneus
19. Rectus abdominis
20. Rhomboideus
21. Sartorius
22. Semitendinosus
23. Serratus dorsalis & Serratus ventralis
24. Triceps lateralis
25. Triceps longus
26. Trapezius

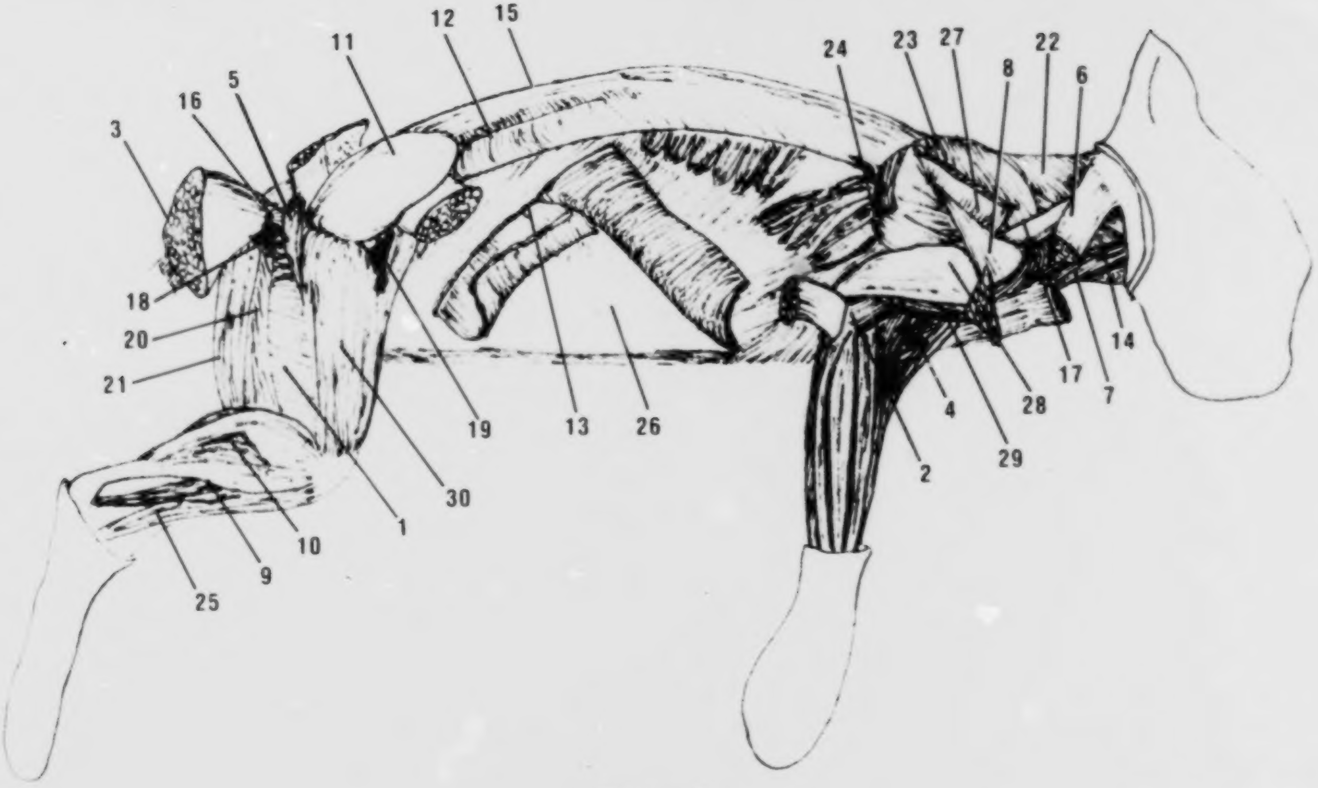
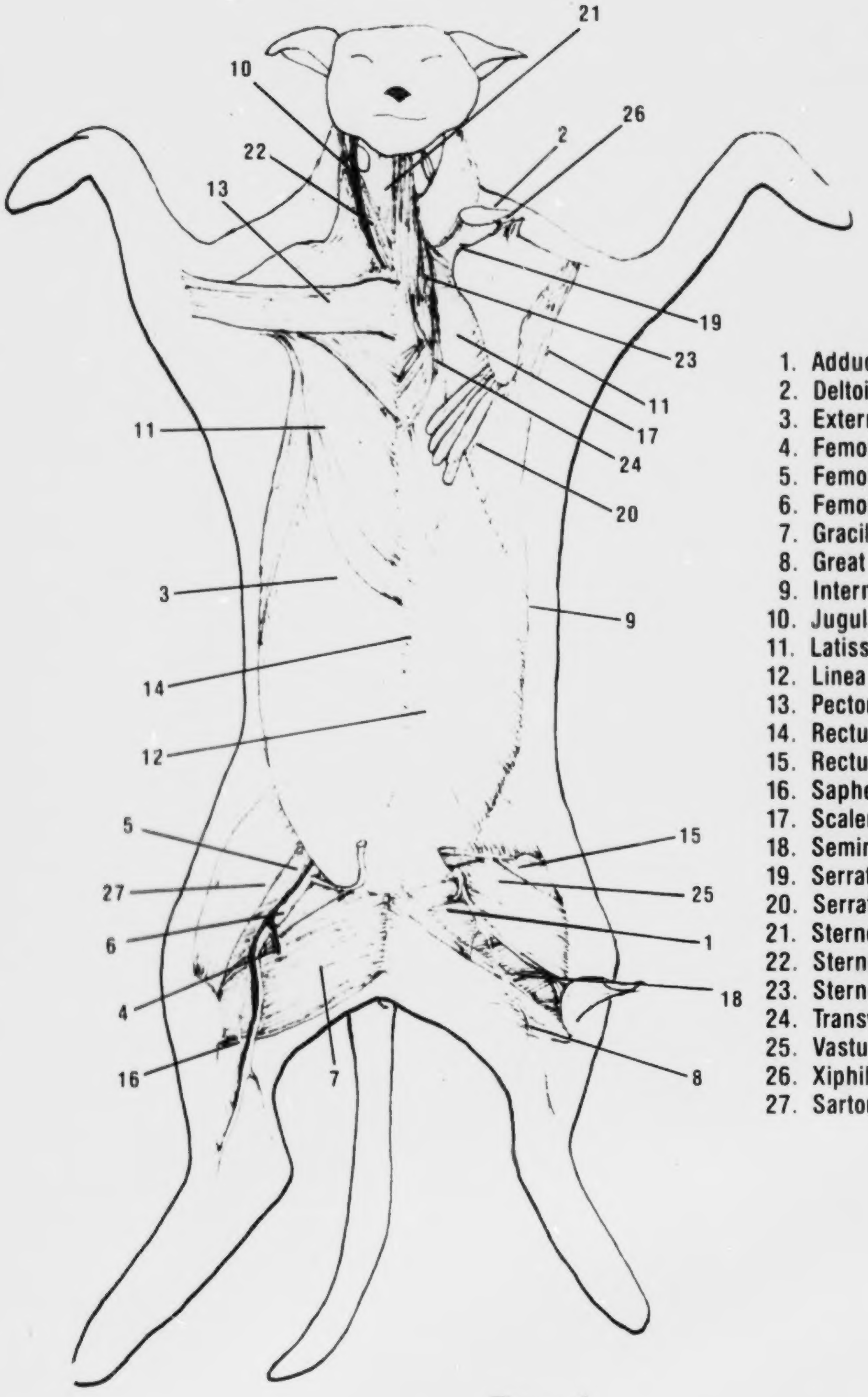


Figure 3

1. Adductor femoris
2. Anconeus
3. Biceps femoris (cut)
4. Brachioradialis
5. Caudofemoralis
6. Cleidocephalicus (cut)
7. Cleidomastoideus
8. Deltoideus - pars scapularis
9. Flexors
10. Gastrocnemius (lateral head)
11. Gluteus medius
12. Iliocostalis
13. Internal oblique
14. Longus capitis
15. Longissimus lumborum
16. Obturator internus
17. Omotransversarius
18. Quadratus femoris
19. Sartorius
20. Semimembranosus
21. Semitendinosus
22. Splenius
23. Supraspinatus
24. Teres major
25. Tibialis cranialis
26. Transversus abdominis
27. Trapezius - thoracica & scapularis
28. Triceps brachii longus
29. Triceps brachii medialis
30. Vastus lateralis



1. Adductor femoris
2. Deltoideus
3. External oblique
4. Femoral artery
5. Femoral nerve
6. Femoral vein
7. Gracilis
8. Great saphenous vein
9. Internal oblique
10. Jugular vein
11. Latissimus dorsi
12. Linea alba
13. Pectoralis
14. Rectus abdominis
15. Rectus femoris
16. Saphenous nerve
17. Scalenus medius
18. Semimembranosus
19. Serratus ventralis cervicis
20. Serratus ventralis thoracis
21. Sternohyoideus
22. Sternomastoideus
23. Sternothyroideus
24. Transversus costarum
25. Vastus medialis
26. Xiphihumeralis (cut)
27. Sartorius

Figure 4

Name	Location	Action
Extensors		
Flexors		
Biceps		
Triceps		
Gastrocnemius		
Latissimus dorsi		
Trapezius		
Deltoids		
Cleidocephalicus		
External oblique		
Gluteus medius		
Semimembranosus		
Splenius		
Tibialis		
Pectoralis		
Sternothyroideus		
Sternomastoideus		
Masseter		
Sartorius		
Rectus femoris		

Figure 5

12. In the chart provided below, figure six, identify other muscles you have found in your specimen.

Name	Location	Action

Figure 6

Procedure: Hour 3

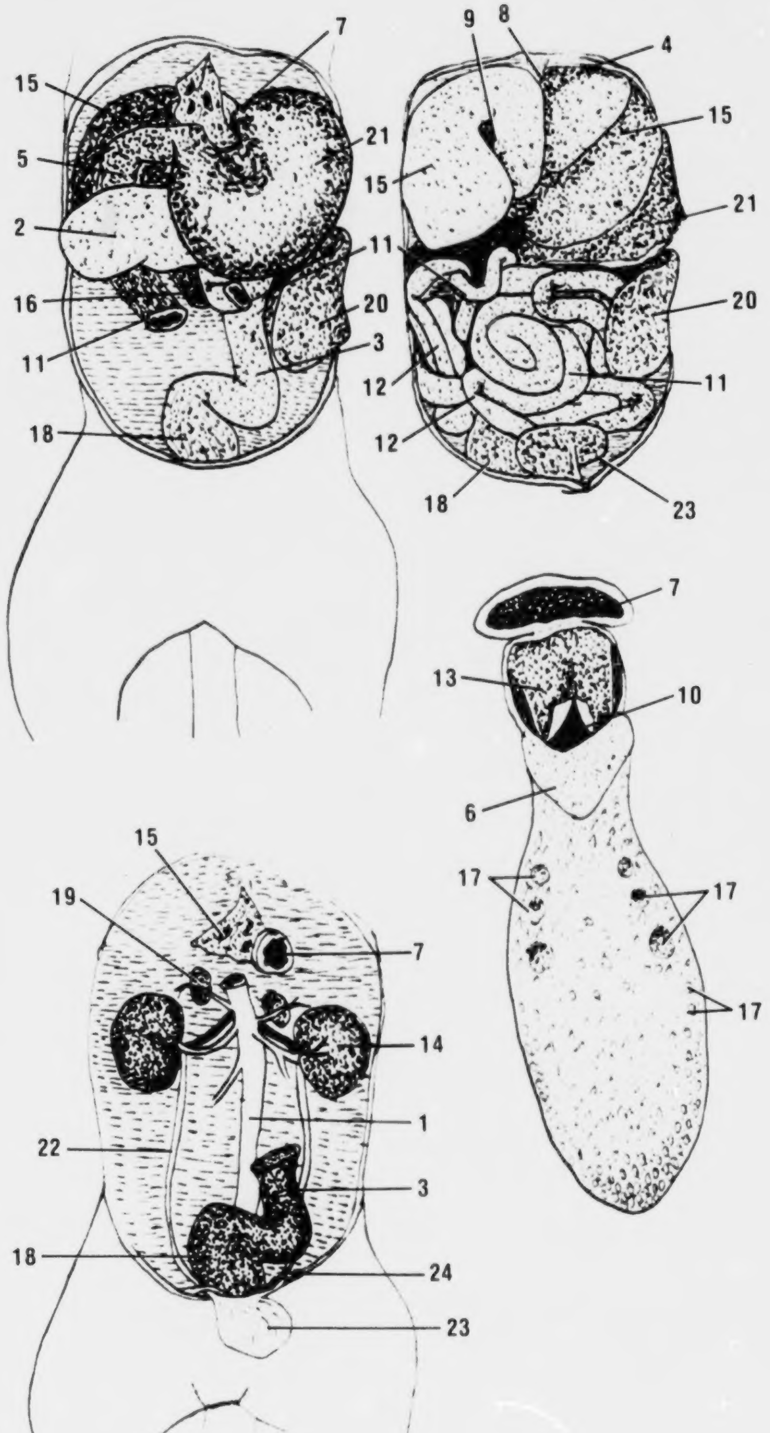
Students need the following supplies: cat, dissecting pan, probe, forceps, dissecting scissors, bone-cutting scissors, colored pencils, and plastic bag and tie.

You will expose and study the abdominal and thoracic cavities and viscera during this laboratory hour, and identify the major visceral membranes and organs of your specimen.

Steps

A. Open the abdominal and thoracic cavities with scissors. Cut through the cat's body wall just to the right of the midventral line from the clavicle to the anus. To do this in the thoracic region, you will have to cut through the cartilagenous ribs on the cat's right side. **Do not** attempt to split the sternum. As you cut through the ribs, make lateral cuts as needed in order to lift the ribs up and out to expose the thoracic cavity. As you lift the ribs up and out, each rib should crack near the vertebral column. If they do not crack, cut them.

The two coelomic cavities, both abdominal and thoracic, lie inside a balloon-like membrane which extends the length of the trunk. This serous membrane derives from a pair of membranes which enclosed organs and tissues in opposite sides of the body, many of which still appear in pairs. However, mammals have evolved so that some organs, such as the stomach and intestines, extend into both sides of the body. The coelomic membranes grew together and became double-walled in places, particularly along the mid-line of the body. These double-walled sections are called mesentery, and they support and protect organs within the coelom.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1. **Abdominal aorta**
2. **Cecum**
3. **Colon**
4. **Diaphragm**
5. **Duodenum**
6. **Epiglottis**
7. **Esophagus**
8. **Falciform ligament**
9. **Gall bladder**
10. **Glottis**
11. **Ileum**
12. **Jejunum**
13. **Larynx**
14. **Left kidney**
15. **Liver**
16. **Pancreas**
17. **Papillae**
18. **Rectum**
19. **Right adrenal**
20. **Spleen**
21. **Stomach**
22. **Ureter**
23. **Urinary bladder**
24. **Vas deferens**

Figure 7

Within the thoracic cavity you should see a double-walled structure surrounding the heart. This double-walled mesentery is called the pericardium. The membranes at the caudel, or lower end of the pericardial sac are thickened with connective tissue which attaches to the diaphragm. The diaphragm effectively divides the coelomic cavities into abdominal and thoracic regions, or, as they are also known, respectively, the peritoneal sac and the pleural and pericardial sacs. Various ligaments attach organs such as the liver, stomach, and reproductive organs to the mesentery in the peritoneal, or abdominal sac.

B. Locate the above mentioned membranes, sacs, and ligaments in your specimen. Figure seven should help you with the identification process.

C. Locate the following thoracic structures in your specimen.

1). The lungs and heart. These organs nearly fill the entire thoracic cavity.

13. Draw the lungs in the space provided below. Include all lobes in your sketch, and label structures to which the lungs are attached.

2). Thymus gland. Locate the thymus between the lungs and above, or cranial to the heart. The thymus covers large blood vessels and the bifurcation of the trachea from the lungs into primary bronchi. The younger the specimen the larger the thymus will be. It may be so large that the bronchi will not be visible until it is removed. The thymus is part of the lymphatic, or immune system, as noted in discussion twenty-two.

14. Draw the thymus in the space provided below. Label the structures to which the thymus gland is attached.

D. Locate the following abdominal structures.

1). Esophagus. From the pharynx the esophagus passes on the ventral side of a cat's neck, through the thoracic cavity dorsal to the trachea, to join the stomach at the cranial end of the abdomen.

2). Liver. The liver is the very large, dark-brown organ pushed against the diaphragm. It is the largest organ in the cat. It is divided into right and left halves. The left liver consists of two lobes and the right liver consists of three. Bile is drained from liver cells by very small canals which run between the individual liver cells. These little canals collect into bile ducts which in turn drain into the gall bladder.

In addition to bile production the liver is responsible for chemical conversions of sugar, amino acids, and fats absorbed from the intestines.

3). Gall bladder. The gall bladder is a dark-green organ stuck in the right lobe of the liver. It is a collecting point for bile. The gall bladder eventually empties its bile collection into the duodenum as it is needed for digestion, primarily of fats.

15. Draw the liver and gall bladder in the space provided below. Include all the lobes, and label the structures to which the liver and gall bladder are attached.

4). Stomach. The stomach is a fat J-shaped structure just below the liver in your specimen. It should be greyish-white in color, and it is divisible into three regions. The first region, called the cardiac region, is where the stomach is hooked in to the esophagus. The large, bulging portion in the middle of the stomach is called the fundic, and the narrow region which joins the small intestine is called the pyloric region.

5). Spleen. The spleen is a long, flat, red or dark-brown organ lying to the left of the stomach. The spleen is part of the lymphatic system, along with the thymus, lymph nodes, and follicles. (See figure fifteen in hour five of this laboratory for a diagram of lymph node locations). In addition to the spleen's role as a lymphatic organ, it removes old red blood cells from circulation, and dissociates their hemoglobin into bilirubin (bile pigment) and iron which are restructured into hemoglobin within new red blood cells.

16. Draw the spleen in the space provided below. Label the structures to which the spleen is attached.

6). Small intestines. These intestines hook onto the stomach. They are attached to each other and the dorsal body wall by mesentery containing blood vessels and nerves. The small intestines are divisible into three sections. Where the intestine hooks onto the stomach is known as the duodenum. The middle portion of the small intestine is called the jejunum, and the last section is called the ileum.

7). Pancreas. The pancreas is a two-lobed gland in the mesentery between the stomach and the loop of the duodenum. The pancreas is usually drained by two ducts. One duct joins the bile duct from the gall bladder and empties into the duodenum. Often there is an additional duct which enters the duodenum near the jejunum.

The pancreas produces seven or eight different digestive enzymes in the cat. Additionally, areas of the pancreas called Isles of Langerhans produce the hormones insulin and glucagon.

17. Draw the pancreas of your specimen in the space provided below. Label the structures to which the pancreas is attached.

8). Cecum. The cecum is a blind pouch where the small and large intestines meet.

9). Large intestine. The large intestine, or colon, like the stomach and small intestines, is divided into three main segments. The first section is on the right side of your specimen and is known as the ascending colon. The middle portion of the large intestine is on the left side of your specimen and is called the descending colon. The intestinal tract which connects the ascending and descending colons is called the transverse colon. In a cat the transverse colon is poorly defined. The last section of the large intestine is a straight tube connected with the anus known as the rectum.

10). Anal glands. The anal glands are paired scent glands opening to the rectum very close to the anus.

18. Draw the anal glands of your specimen in the space provided below. Label the structures to which they are attached.

11). Kidneys. The kidneys are dark red-brown structures the size of walnuts which are located on the dorsal abdominal wall. Unlike the other organs observed so far, the kidneys are not suspended in the abdomen but are attached on either side of the backbone. They are covered by the peritoneum, and the right kidney should be touching the liver.

12). Adrenal glands. The adrenals are located on the cranial side of each kidney, like a cap on top of each organ.

19. Draw the kidneys and adrenal glands in the space provided below. Label the structures to which they are attached in the body of your specimen.

Procedure: Hour 4

Students need all listed supplies except the straw.

During this laboratory hour you will trace the digestive and respiratory systems of your specimen.

Steps

A. Locate and study the salivary glands. There are five pairs of salivary glands, three pairs of which are easy to see and should have been exposed when you removed the skin of the head and neck to examine the cat's musculature. The major function of salivary glands is to provide lubrication for food. Secondarily they secrete the enzyme amylase which converts starch to dextrose.

The first pair to be studied, the parotid glands, lie just beneath the skin and below the ear. The parotid ducts connecting them to the mouth open just inside the cheek opposite the last premolar tooth. The parotids are composed of predominantly enzyme-producing cells which secrete amylase.

The submaxillary glands are below the parotids at the angle of the jaw. The submaxillary ducts connecting the glands to the mouth's interior open to the mouth through a small papilla at the base of the cat's tongue. The submaxillary glands are composed equally of enzyme producing cells and mucus producing cells. The sublingual glands are attached to the submaxillary glands and are about two centimeters long. They are composed almost exclusively of mucus producing cells.

20. Diagram in the space provided below the location, position, and over-all shape of the parotid, submaxillary, and sublingual salivary glands in the cat's head.

B. Cut through the muscles and skin at the corners of your specimen's mouth. Press down on the lower jaws with your fingers, being careful not to impale yourself on cat teeth. Cut the angle of the jaw with bone cutting scissors. Locate and identify the following structures:

1). Cheeks and lips.

2). Vestibule. The vestibule is the space between the lips and teeth.

3). Teeth.

4) Tongue. Note the taste buds, or papillae, on the upper surface of the tongue. Note that almost the entire tongue is a muscle and the variety of movements possible with it. The tongue is one of the most complex components of the muscular system.

5. Hard and soft palates. The hard palate is the roof of the mouth; the soft palate hangs from the back part of the hard palate. Nasopalatine ducts (naso means "from the nose") open through the front section of the hard palate. Above and at the end of the hard palate and continuing across the soft palate is part of the pharynx.

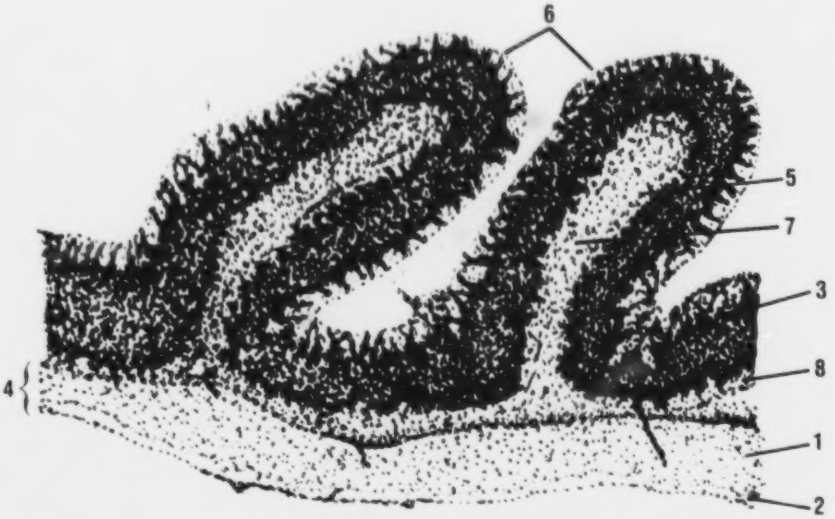
C. Study the pharynx of your cat. The pharynx is the crossing of air and food passages in an animal. Openings from the nares, ears, mouth, lungs, and stomach all converge in the pharynx.

The glottis is a flap of tissue in the pharynx which functions to direct food from the mouth into the esophagus and on to the stomach, or air into the trachea past the glottis and voicebox, or larynx, and on to the lungs.

Split the soft palate of the cat for the best observation of the cavity called the nasopharynx in which the two eustachian tubes leading from the middle ear cavities open along the lateral walls.

You will also see tonsils in the pharynx. They are lymphatic nodules located just past the base of the tongue.

D. The esophagus conveys food from the pharynx to the stomach. Study the cat stomach, with the aid of the drawing in figure eight below, which depicts an enlarged cross-section view of the stomach.



1. **Circular**
2. **Longitudinal**
3. **Mucous cells**
4. **Muscularis externa**
5. **Muscularis mucosa**
6. **Rugae**
7. **Submucosa**
8. **Zymogen cells**

Figure 8

The cardiac stomach, the section attached to the esophagus, has deep-branching, columnar mucus glands. The fundus, or middle stomach, has deep gastric pits lined with cuboidal cells. The cells lining the upper half of the pits are mucus producing chief cells, and those lining the lower half of the pits secrete zymogen, an enzyme precursor which is activated by acidic catalysis. Layered under the chief cells are parietal cells which secrete hydrochloric acid. The pylorus, or lower stomach, has deep-branchng mucus glands like the cardiac stomach.

E. Take a minute cross-section of the stomach lining, and mount it on a slide with coverslip. Observe this cross-section mount under both low and high power of the microscope.

21. Draw your stomach lining cross-section in the space provided below. Label all the cell types visible in the slide.

22. From which stomach section did you take your specimen? ____________________

23. How could you have identified the stomach section from which the specimen had been taken without knowing in advance where it came from?

____

____

24. From which stomach section was the figure eight drawing taken? ____________________

25. Draw the entire stomach of your specimen in the space provided below, with its attachments to the esophagus and duodenum. Label the sections of the stomach.

F. Cut the small intestine where it joins the stomach. Cut away the mesentery which connects the small intestine loops and attaches it to the peritoneal membranes. Leave it attached to the large intestine, stretch it out from the body cavity, and measure the small intestine.

26. How long is the small intestine of your specimen? ______________________________

The duodenum, which attaches to the stomach, is characterized by the presence of mucus secreting glands called Brunner's glands. In cross-section the lining of the duodenum would appear as in the figure nine drawing.

1. **Brunner's glands**
2. **Circular**
3. **Longitudinal**
4. **Musculars externa**
5. **Submucosa**
6. **Villus**

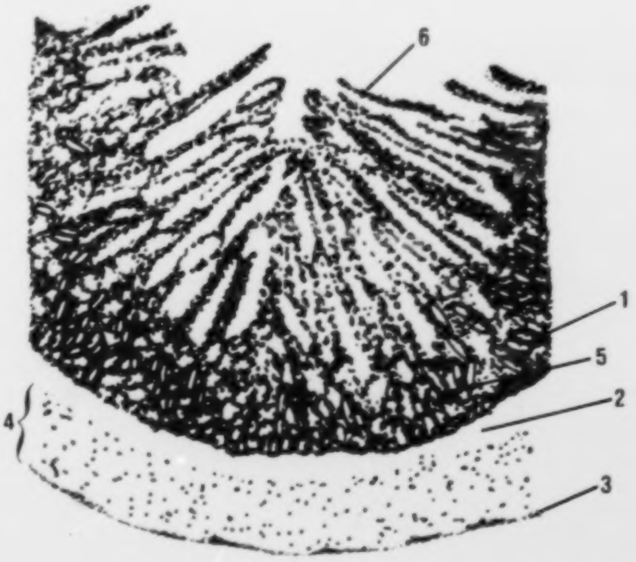


Figure 9

G. The ileum, the final section of the small intestine, is characterized by groupings of lymphatic nodules called Peyer's patches. Cut a minute cross-section of ileum lining and mount it on a slide with coverslip. Examine it under both low and high power of the microscope.

27. Draw in the space provided below what you see in the ileum lining cross-section mount. Label what you can identify.

H. Study the colon. The entrance to the colon from the ileum is guarded by a sphincter valve, the ileocolic sphincter. Cut the colon where it joins the ileum and identify the sphincter.

28. In what direction does the ileocolic sphincter allow material to pass? ______________________________

29. In the space provided below draw the colon as it lies in the cat. Label all sections of the colon, and show its attachments.

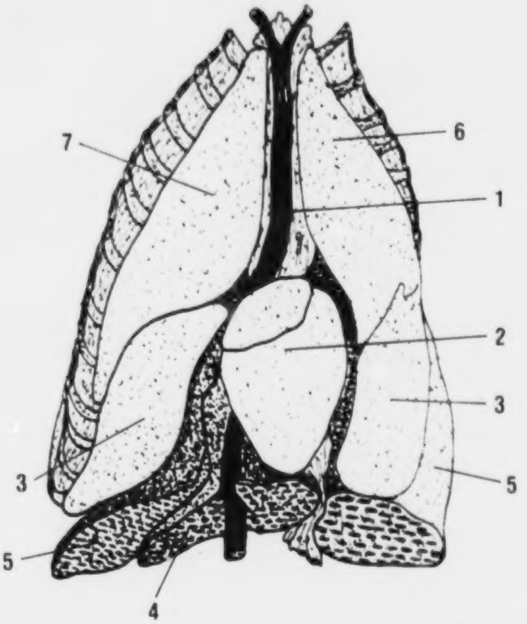
Procedure: Hour 5

Students need all listed supplies, except the straw.

During this laboratory hour you will identify and trace the respiratory and circulatory systems of the cat.

Steps

A. Completely expose the thoracic cavity. Using bone cutting scissors, cut through the cartilage on both sides of the sternum. Make an incision at right angles to the sternum between two caudal ribs. Bend back the thoracic walls and break or cut the ribs near the spinal column. This will expose the pleural cavity and the lungs. Figure ten on the following page diagrams the thoracic viscera you should see.



1. Cranial vena cava
2. Heart
3. Lung: cardiac lobe
4. Lung: caudate lobe
5. Lung: diaphragmatic lobe
6. Lung: left apical lobe
7. Lung: right apical lobe

Figure 10

The pleura is the lining of the thoracic cavity and the lung. The parietal pleura lines the cavity, while visceral pleura covers the surface of the lungs themselves.

B. Review the respiratory structures studied during hour three, and define those terms listed below by describing their placement and function in the cat:

30. Nares

31. Nasopharynx

32. Pharynx

33. Glottis

34. Larynx

35. Trachea

Bronchii branch from the trachea and attach to the lungs, one bronchus, or tube going to each lung. Within the lungs, the bronchii subdivide into bronchioles, ultimately terminating in small chambers called vestibules which are bordered by alveoli pockets.

36. The larynx, trachea, and bronchii are all stiffened by support tissue. What is this support tissue?

____

The lungs of a cat occupy most of the thoracic cavity. The right lung is composed of four lobes and the left lung has three lobes.

C. Section a minute piece of lung tissue and mount it on a slide with coverslip. Observe it under both low and high power of the microscope.

37. In the space provided below, draw a clear view of your lung tissue slide. Label all identifiable structures, such as alveoli, vestibules, and bronchioles.

D. Using probe and forceps, open the pericardial sac to expose the heart and blood vessels. Notice that the pericardium is composed of two membranes, a parietal and a visceral pericardium, similar in structure and function to those encompassing the lungs. Locate and study the following structures.

1). Left and right ventricles. The ventricles are the chambers at the lower apex of the heart.

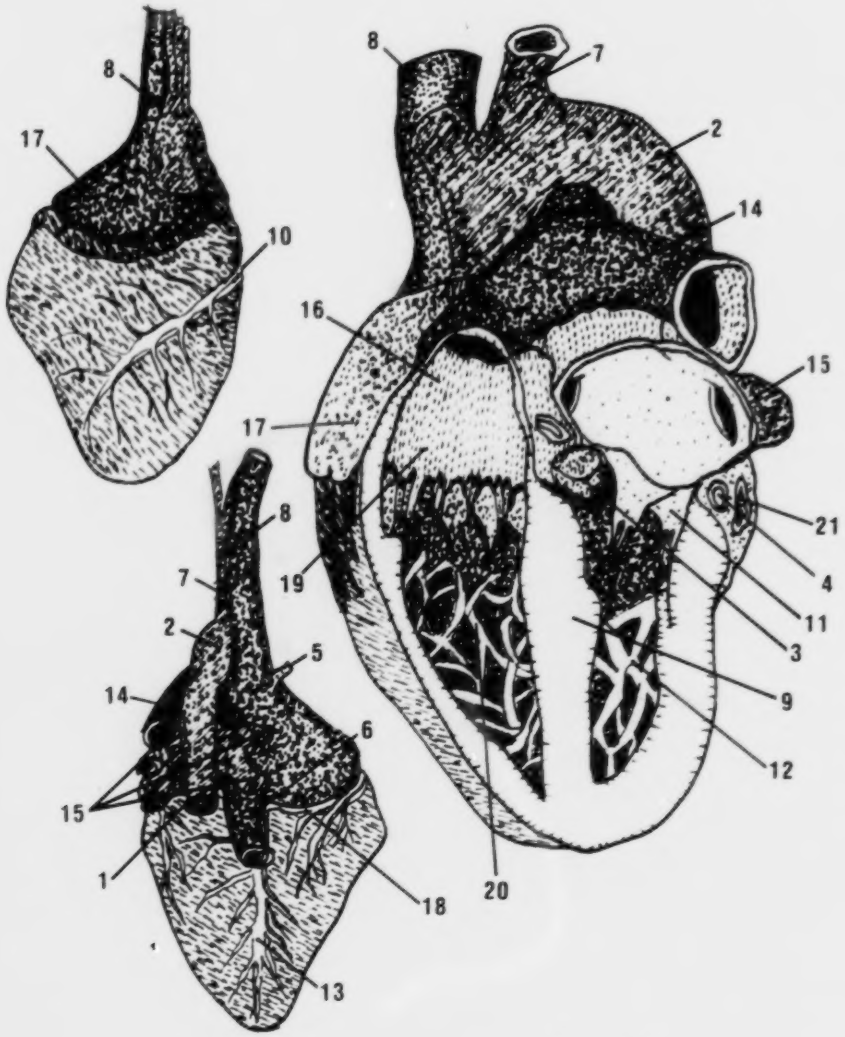
2). Left and right atria. The atria are thinner walled chambers which sit like caps on top of the ventricles. Covering the atria are small muscular lobes, called auricles, which extend downwards over part of each ventricle.

3). The pulmonary arch. The pulmonary arch passes between the two auricles, and branches into right and left pulmonary arteries, which further subdivide before reaching the lungs.

4). The aorta. The aorta is a large arch beginning at the left ventricle. If your specimen is fat, it may be necessary to remove some fatty deposits before the aorta is visible.

5). Veins. Caudal and cranial vena cavae enter the right atrium. Pulmonary veins enter the left atrium.

The following figure eleven diagram will aid in your study of the cat's heart.



1. Aorta
2. Aortic arch
3. Aortic semilunar valve
4. Artery
5. Azgos vein
6. Caudal vena cava
7. Common carotid artery
8. Cranial vena cava
9. Interventricular septum
10. Left coronary artery & great cardiac vein
11. Left tricuspid valve
12. Left ventricle
13. Middle cardiac vein
14. Pulmonary artery
15. Pulmonary vein
16. Right atrium
17. Right auricle
18. Right coronary artery
19. Right tricuspid valve
20. Right ventricle
21. Vein

Figure 11

E. Cut the pulmonary arch, the vena cavae, the pulmonary veins, and the aortic arch close to the heart and remove the heart. Loosen and then pull out the latex probably remaining in the stub of the aortic arch of your cat's heart. The latex will contain a negative impression of the semilunar valve of the aorta. Slice the heart transversely through the wall of each atrium down to the apex of the ventricles. Using forceps, wash and pick out the clotted blood. Locate and identify the following structures.

1). Tricuspid valve. This valve prevents blood in the right ventricle from entering the right atrium. The flaps of the valve are held to the walls of the right ventricle by chordae tendinae, which are attached by raised bundles of muscle. Even though tricuspid valves have three flaps (hence the name), the flaps are difficult to distinguish in a cat.

2). Pulmonary semilunar valve and aortic semilunar valve. These valves are found between the pulmonary arch and the right ventricle, and between the aortic arch and the left ventricle, respectively. Note their shape, and compare them with the negative impression in the latex pulled from the stub of the aortic arch.

38. Draw the pulmonary and aortic semilunar valves in the space provided below.

39. Are they similar? ______________

3). Bicuspid valve. This valve is similar to the tricuspid, except that it has two flaps instead of three. The two flaps should be visible in your specimen.

40. In the space provided below, draw both the bicuspid and tricuspid valves of your cat's heart. Label them.

41. Are they similar? ______________

F. Study the figure twelve diagram of cat arteries. Using probe and forceps, identify the major arteries of the cat specimen.

1. **Abdominal aorta**
2. **Adrenolumbar artery**
3. **Aorta**
4. **Caudal artery**
5. **Caudal adrenal artery**
6. **Caudal mesenteric artery**
7. **Caudal phrenic artery**
8. **Caudal vena cava**
9. **Celiac artery**
10. **Cranial abdominal artery**
11. **Cranial adrenal artery**
12. **Cranial mesenteric artery**
13. **Cranial phrenic artery**
14. **External iliac artery**
15. **External jugular**
16. **Femoral artery**
17. **First rib**
18. **Iliolumbar artery & vein**
19. **Intercostal artery**
20. **Internal ilial artery**
21. **Kidney**
22. **Left subclavian artery**
23. **Ovary**
24. **Ovarian vein**
25. **Renal artery**
26. **Urinary bladder**
27. **Uterus**
28. **Thoracic aorta**

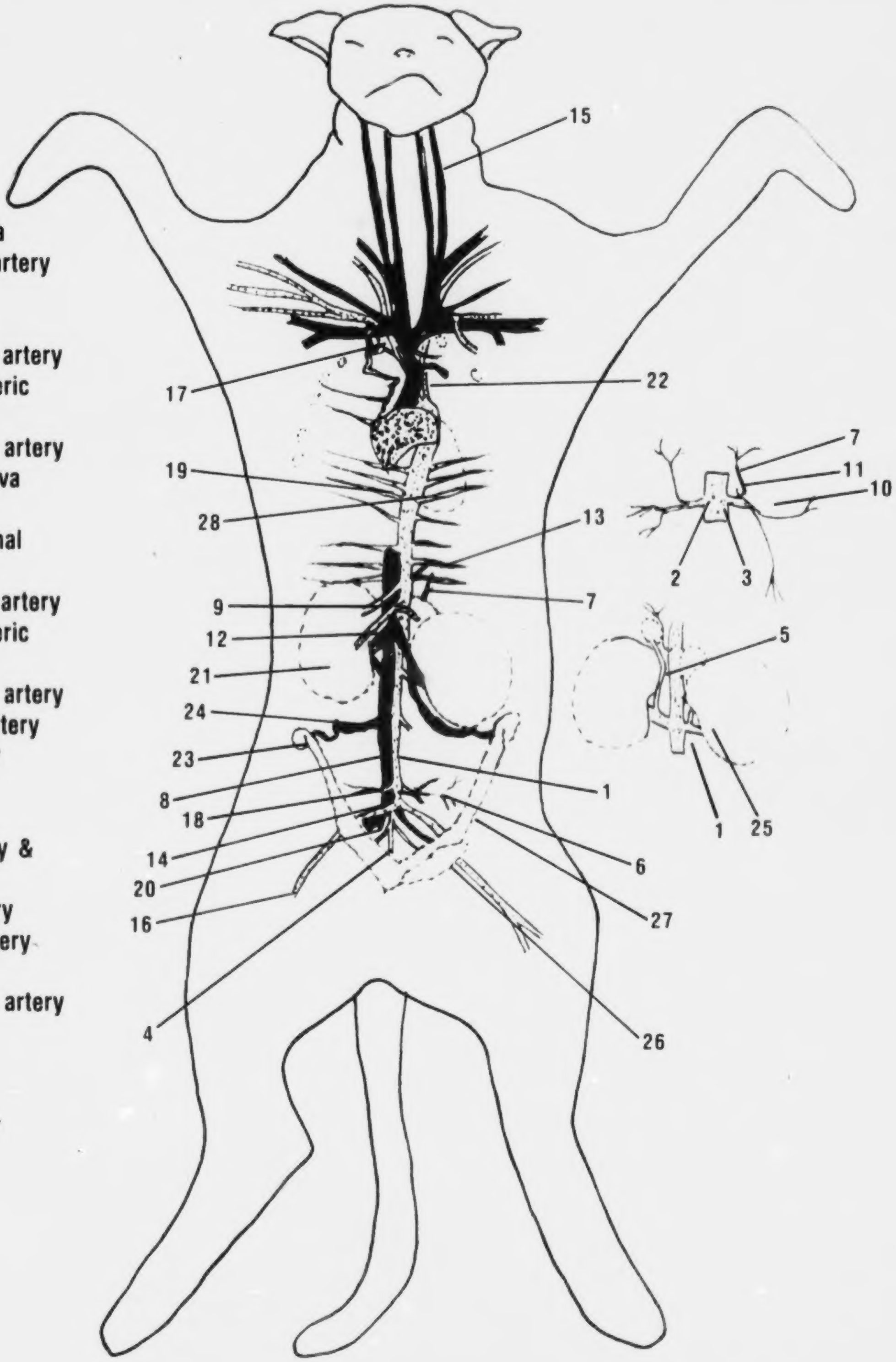


Figure 12

42. Complete the figure thirteen chart below.

Artery	Location	Origin
Innominate		
Carotid		
Cranial thyroid		
Lingual		
Celiac		
Renal		
Iliac		
Femoral		
Lumbar		
Gonadal		

Figure 13

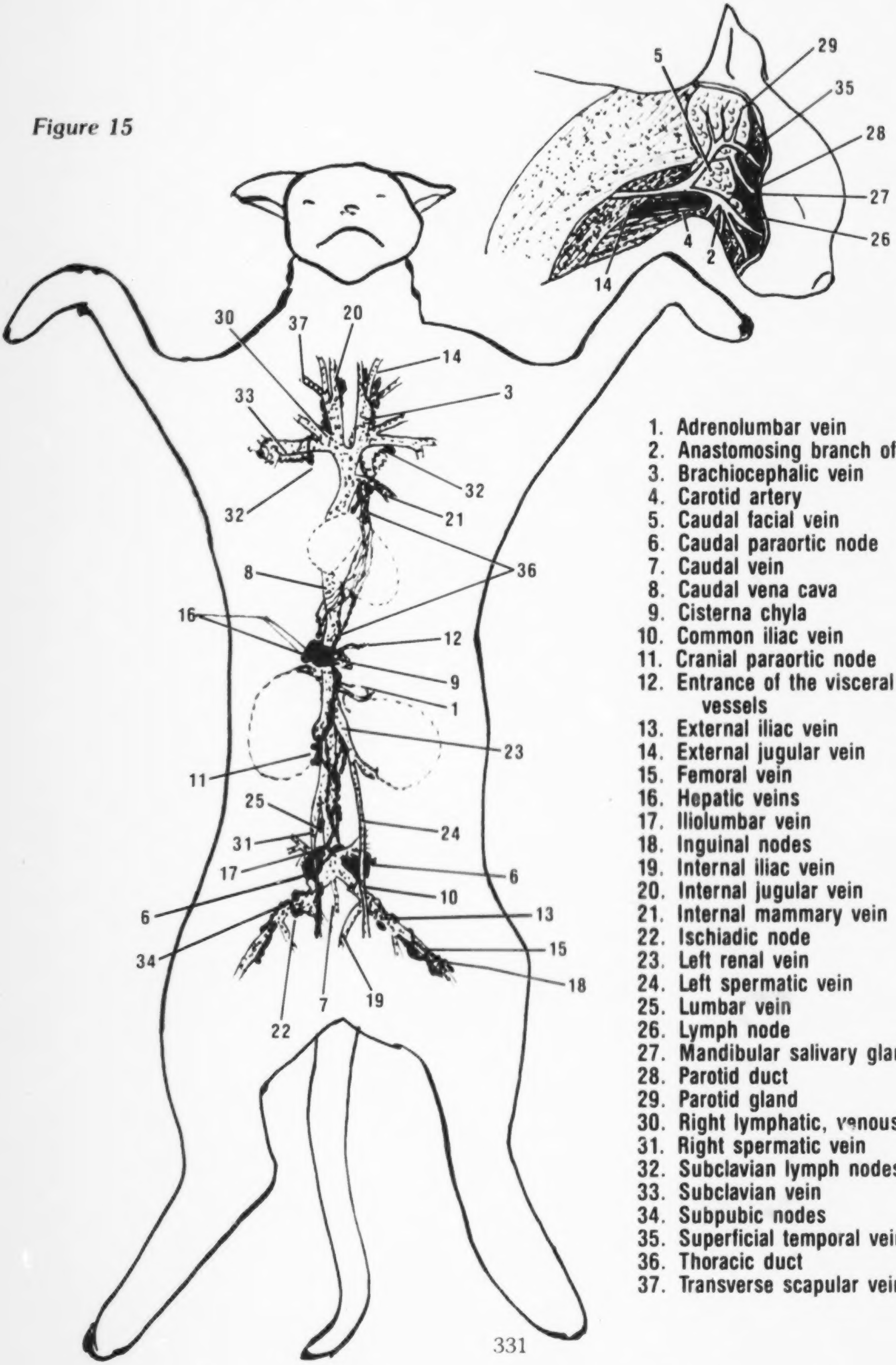
G. Study the figure fifteen diagram (on following page) of veins and glands in a cat. Using probe and forceps, identify the major veins and glands of your specimen. Very little dissection will be necessary to expose the veins, since they usually accompany the arteries.

43. Complete the figure fourteen chart below.

Vein	Location	Origin
Vena cava		
Azygos		
Mammary		
Phrenic		
Iliac		
Mesenteric		
Jugular		
Cephalic		
Hepatic		
Renal		

Figure 14

Figure 15



1. Adrenolumbar vein
2. Anastomosing branch of the jugular
3. Brachiocephalic vein
4. Carotid artery
5. Caudal facial vein
6. Caudal paraortic node
7. Caudal vein
8. Caudal vena cava
9. Cisterna chyla
10. Common iliac vein
11. Cranial paraortic node
12. Entrance of the visceral lymph vessels
13. External iliac vein
14. External jugular vein
15. Femoral vein
16. Hepatic veins
17. Iliolumbar vein
18. Inguinal nodes
19. Internal iliac vein
20. Internal jugular vein
21. Internal mammary vein
22. Ischiadic node
23. Left renal vein
24. Left spermatic vein
25. Lumbar vein
26. Lymph node
27. Mandibular salivary gland
28. Parotid duct
29. Parotid gland
30. Right lymphatic, venous union
31. Right spermatic vein
32. Subclavian lymph nodes
33. Subclavian vein
34. Subpubic nodes
35. Superficial temporal vein
36. Thoracic duct
37. Transverse scapular vein

Procedure: Hour 6

Students need all listed supplies.

During this laboratory hour you will locate and study the urinary and reproductive organs of a cat.

Steps

A. Locate the kidneys embedded in fat on the dorsal body wall. Remove the fat surrounding the kidneys, without damaging the adrenals and blood vessels in the area. Note where the ureters attach to the kidneys. Cut one ureter as it exits a kidney, and cut the blood vessels that attach to the kidney. Remove the kidney from the body cavity. Longitudinally slice the kidney in half, in order to see the following sections.

1). Adrenal gland. Review hour three procedure.

2). Cortex. The cortex is the outer region of the kidney which contains blood filtering mechanisms. It should be distinguishable by color from the inner kidney region, the medulla.

3). Medulla. The medulla is the inner region of the kidney. It contains the collecting ducts which carry urine from the cortex. The ducts merge into larger ducts and empty into the funnel-shaped renal pelvis.

4). Renal pelvis. The renal pelvis is a collecting area for urine just before it is funneled into the ureter.

5). Hilus. The hilus is the region where blood vessels enter and leave the kidney.

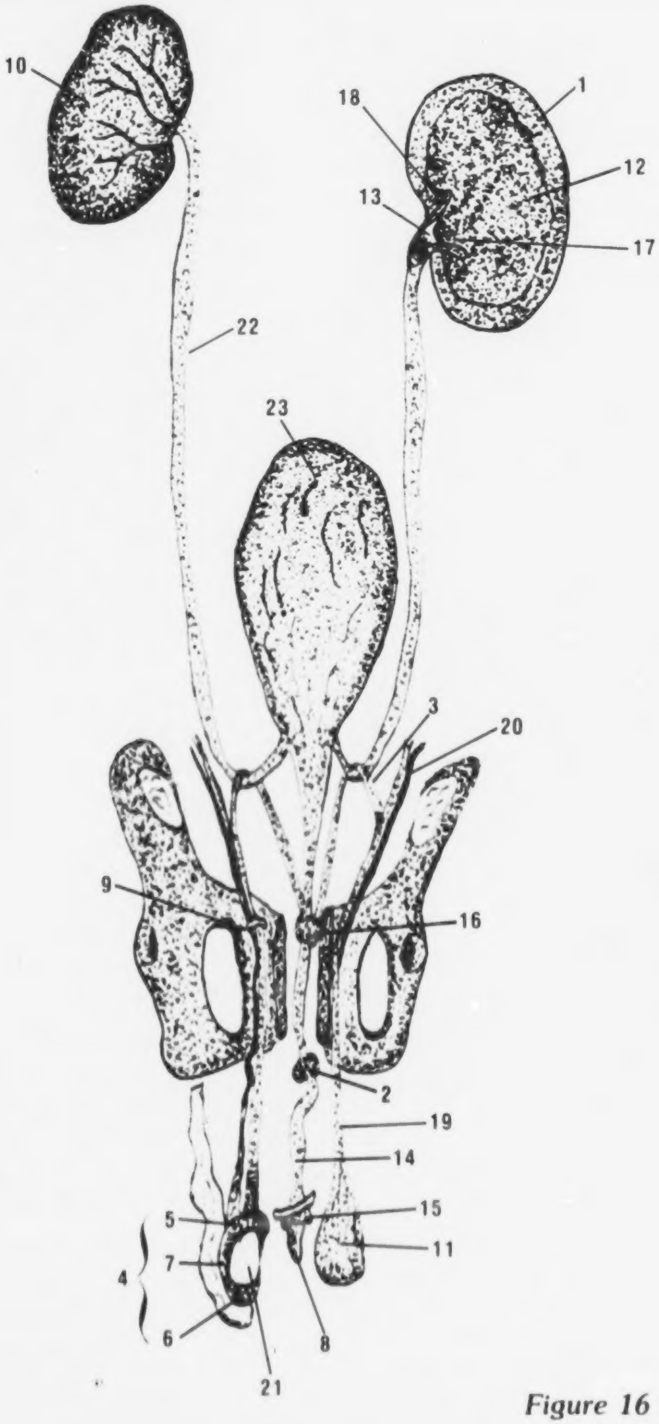
44. In the space provided below, draw your cat's sectioned kidney, and label all parts.

B. Take a straw and place one end in the cut ureter left in the cat's body cavity. Gently blow into the straw. The urinary bladder should swell like a balloon, making it easy to distinguish.

The bladder is a reservoir for urine discharged through the urethra. Because it is held to the ventral body wall by a mesentery suspensory ligament, it might be difficult to find because, in exposing the body cavity, the suspensory ligament was probably cut, thus collapsing the bladder.

C. Depending upon the sex of your cat, identify the following reproductive organs. When you have completed this reproductive dissection, compare cats with another laboratory group which has a cat of the opposite sex. In this fashion you should complete both male and female reproductive sections of this laboratory.

Male Organs: with the aid of figure sixteen, identify the following structures.



1. Cortex
2. Cowper's gland
3. Ductus deferens
4. Epididymus
5. Caput
6. Cauda
7. Corpus
8. Glans penis
9. Inguinal canal
10. Kidney
11. Left scrotum (skin removed)
12. Medulla
13. Pelvis
14. Penis
15. Prepuce
16. Prostate gland
17. Renal papilla
18. Sinus
19. Spermatic cord
20. Spermatic vessel & nerve
21. Testis
22. Ureter
23. Urinary bladder

Figure 16

1). Scrotum. The scrotum is a sac of muscle and connective tissue on the outside of the body, just ventral to the anus, which surrounds the testes. All mammals carry the scrotum on the outside of the body, so that the testes will not overheat, because sperm remain alive only at a temperature lower than body temperature. Remove the scrotum from the cat's body, and slice it in half. Find the testes, paired reproductive glands within the scrotum. Find the coiled epididymis, leading into the spermatic cord which extends from the testes into the abdominal cavity of the cat. The spermatic cord is composed of a duct from the epididymis, spermatic artery and vein, and a nerve, bound together in a sheath.

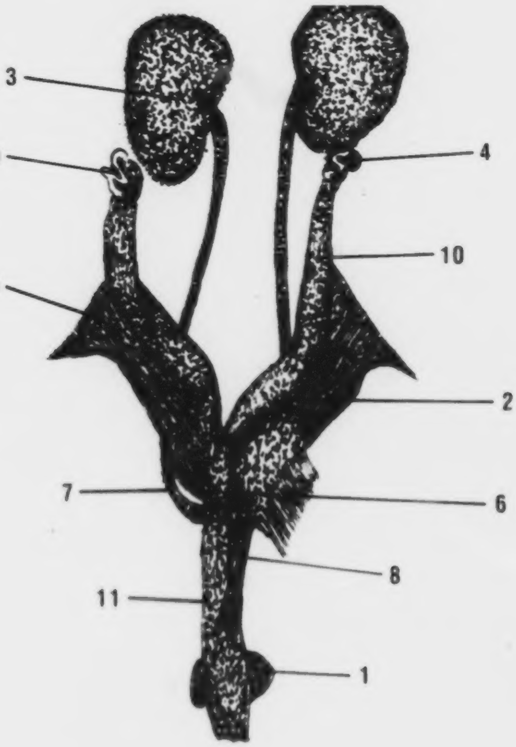
45. Draw a sectioned view of the cat's scrotum in the space provided below. Label all structures exposed in your dissection.

2). Prostate glands. The prostates are small bulb-shaped glands covered by striated muscles. They are located on either side of the urethra near the entrance of an epididymal duct, the ductus deferens. The prostate orchestrates the release of either semen (sperm) or urine from the penis.

3). Penis. The enlarged distal end is called the glans. A male cat has a baculum, or penis bone. Remove the penis and cross-section it. In the center area you should see three cavernous bodies. These sinuses are what engorge with blood when the male cat is sexually aroused. On either side of the penis where muscles attach to the penis, you should see two small glands, Cowper's glands. Cowper's glands discharge stored sperm into the urethral tubes.

46. Diagram and label the structures you have located in the cat's penis in the space provided below.

Female Organs: with the aid of figure seventeen, identify the following structures.



1. **Anal gland (dorsal to vestibule)**
2. **Broad ligament**
3. **Kidney**
4. **Ovary.**
5. **Round ligament**
6. **Suspensory ligament**
7. **Ureter**
8. **Urethra**
9. **Uterine tube**
10. **Uterus**
11. **Vagina**

Figure 17

1). Ligaments. There are several broad ligaments supporting female reproductive organs, such as the ovaries, uterine tubes, and uterus. These ligaments are attached to the dorsal body wall and should still be in place. Another ligament supports the uterus ventrally (although it was cut when opening the cavity), and small ovarian ligaments hold the ovaries in place.

2). Ovaries. Ovaries are the female reproductive glands. The eggs develop in the ovaries by meiotic divisions inside fluid-filled chambers called follicles. The follicles enlarge as the eggs within them mature. Mature follicles are known as Graafian follicles. After the egg erupts (ovulation), the walls, or scar tissue of the erupted follicle, become the corpus luteum. Eventually the corpus luteum degenerates. The corpus luteum produces female sex hormones. Slice a minute section from one ovary, and mount it on a slide with a coverslip. Study this section under both low and high power of a microscope.

47. Draw and label what you see in the cat's sectioned ovary in the space provided below.

3). Uterus. Uterine tubes (oviducts) connect the cat's ovaries to the uterus. The cat uterus has two sections or horns, called cornu, joined caudally, just before the terminal chamber of the female reproductive tract, the vagina. Use bone cutting scissors to cut away the bone (the ischium and the pubis) overlying these organs.

4). Vestibule. The vagina and the urethra both empty into the vestibule, a chamber common to both the reproductive and excretory systems. Dorsal to the vestibule are the anal glands which scent the female. In the floor of the vestibule near the opening of the urethra you will find the clitorus. The clitorus is the counterpart of the male penis, and it is composed of two sinus bodies and a small bone, the os clitoris. It is difficult for a beginner to dissect a cat's clitorus. The walls of the external opening of the vestibule are raised into flaps called vulvae.

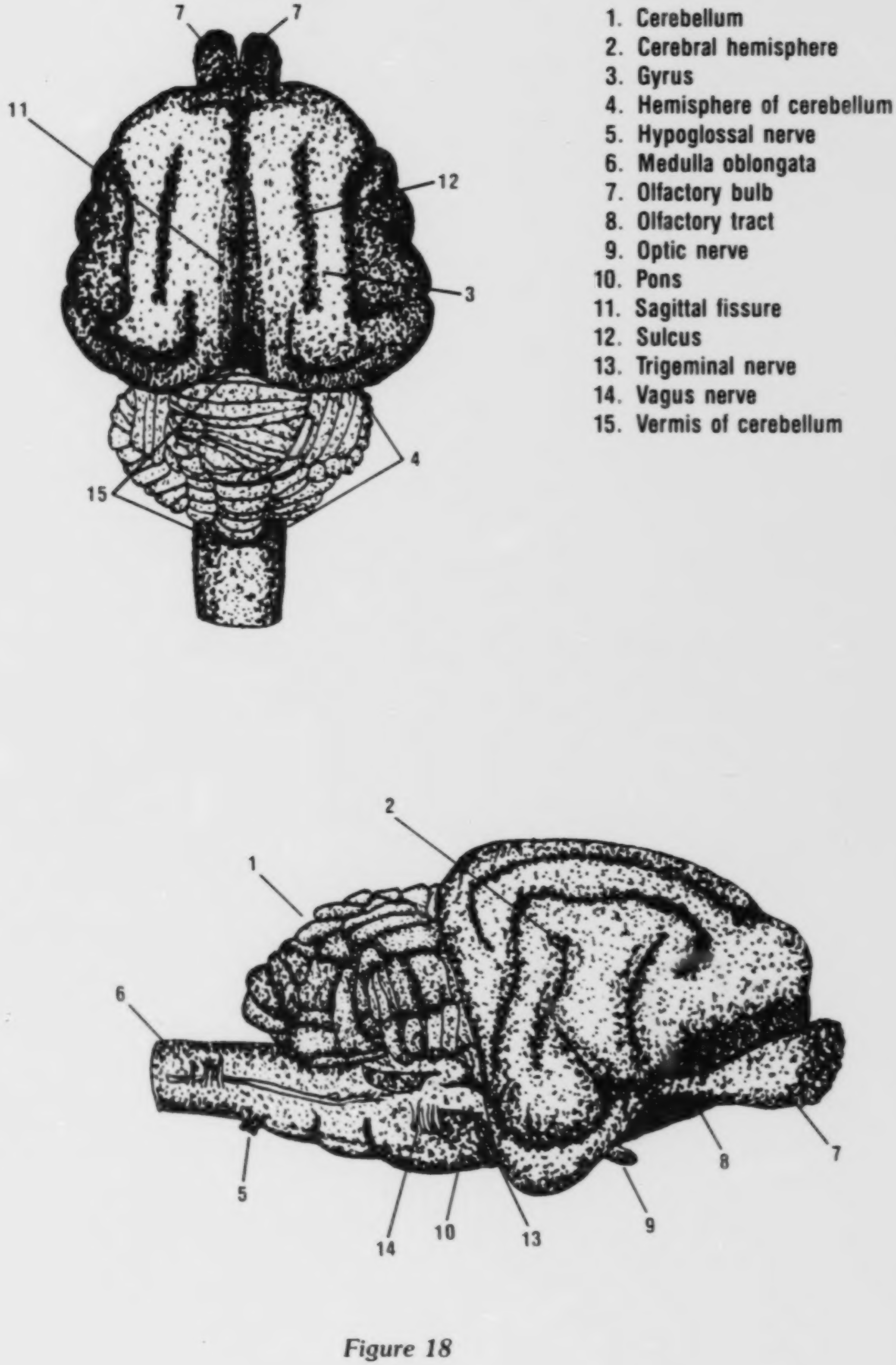
Procedure: Hour 7

Students need all listed supplies, except the straw.

During this laboratory hour you will identify and trace the nervous system of a cat.

Steps

A. Use bone cutting scissors to cut and chip your way into the cranial cavity of your specimen. Older cats have harder crania than younger ones, and some students may need to ask an industrial arts student or instructor for use of an electric saw. Be careful not to cut into the brain itself. After opening the cranium, chip off the remaining roof of the cranium with forceps. Leave the eyes and ears intact for future study. Use figure eighteen to locate and study the following structures.



1. Cerebellum
2. Cerebral hemisphere
3. Gyrus
4. Hemisphere of cerebellum
5. Hypoglossal nerve
6. Medulla oblongata
7. Olfactory bulb
8. Olfactory tract
9. Optic nerve
10. Pons
11. Sagittal fissure
12. Sulcus
13. Trigeminal nerve
14. Vagus nerve
15. Vermis of cerebellum

Figure 18

1). Meninges. The meninges are membranes that cover the brain. All mammals have three layers of membranes. The outermost layer is called the dura mater. The arachnoid, or middle, layer is a network of thin delicate fibers. The innermost layer is called the pia mater; it is stuck to the brain itself.

2). Olfactory bulb. The olfactory nerves terminate here after their journey from the nares.

3). Cerebrum. The cerebrum, or center of intelligence, consists of two halves, or hemispheres, divided by a deep groove called the sagittal fissure. The floor of the sagittal fissure is called the corpus callosum. The corpus callosum is a band of nerve fibers connecting the left and right cerebral hemispheres.

4). Cerebellum. The cerebellum is a very convoluted part of the brain. It is caudal, or behind, the cerebrum. There are two pairs of lobes between the cerebrum and cerebellum called the corpora quadrigemina. They serve as optic reflex centers for the cat.

5). Medulla oblongata. The medulla oblongata is the most caudal portion of the brain, and constricts into the spinal cord.

It is difficult to remove the brain from the cranium without destroying the parts you wish to study. The instructor and/or students may wish, as a demonstration or optional activity, to remove the brain from the cranium. Slice both ventral and sagittal sections of the brain and study what is exposed.

B. Study the cat's eye. Notice the upper and lower eyelids, and a small fold of tissue at the corner of the eye. A very thin membrane, the conjunctiva, covers the exterior of the eyeball and the inner margin of the eyelids. Remove the eyelids and the skin around the eye as far as the ear. Locate the following external structures (in addition to those studied during hour one):

1). Eye muscles. Notice that the muscles attached to the eye, with one exception, all originate near one spot. This spot also is where the optic nerve attaches the eye to the brain.

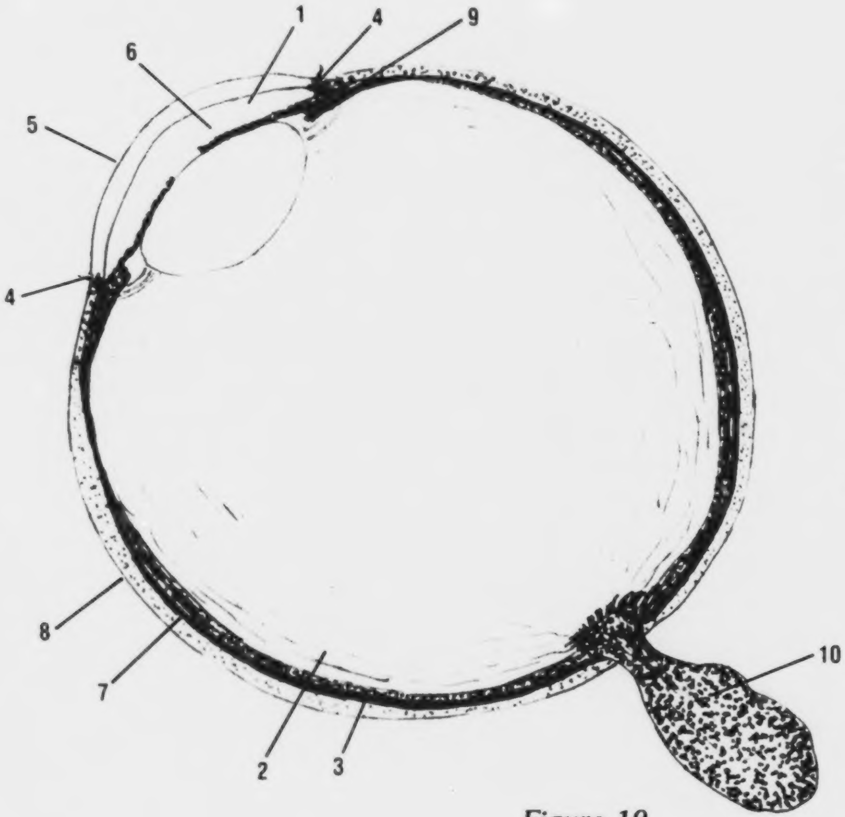
2). Lacrimal glands. These are found near the corner of the eye.

3). Harderian gland. The Harderian gland is a C-shaped gland surrounding the front, or external, part of the eyeball.

48. In the space provided below, draw the eye so as to show muscles and glands. Label these structures.

C. Remove the eyeball from the optic foramen (the orbit of the cranium in which it rests) by cutting the eye muscles and optic nerve. Locate the sclera, the tough, white, fibrous outer layer of the eyeball, and the cornea, the exposed transparent continuation of the sclera.

D. Cut the eyeball in half in a plane parallel to the optic nerve and locate the following. Refer to figure nineteen for help in identification.



1. **Anterior chamber**
2. **Area of tapetum lucidum**
3. **Choroid**
4. **Ciliary body**
5. **Cornea**
6. **Iris**
7. **Retina**
8. **Sclera**
9. **Suspensory ligament**
10. **Optic nerve**

Figure 19

1). Blind spot. The blind spot is where the optic nerve enters the retina.

2). Retina. The retina is the light sensitive, yellow tissue which coats the interior chamber of the eye. The retina is a complex tissue layer composed of rods and cones (special cells for sight and color), nerve fibers, and pigments. The fovea centralis is a specialized area of the retina directly behind the pupil. The interior chamber of the eye is filled with vitreous humor, a jelly-like substance which provides shape to the eye. The vitreous humor clings together in a vitreous body, and it probably fell out of the eye when you sliced into it.

3). Choroid. Underneath the retina of the eye is the choroid layer. After the choroid layer is the sclera. Choroid tissue is black except for the portion directly behind the pupil, which is a metallic blue-green. This area is called the tapetum lucidum. The tapetum reflects light back through the retina and out the pupil. The ciliary body is an enlargement of the choroid between the iris and choroid proper. The ciliary body contains a muscle which controls eye lens movement. The iris is also a continuation of the choroid. The iris is a pigmented partition pierced by the pupil. It separates the anterior and posterior chambers of the eye.

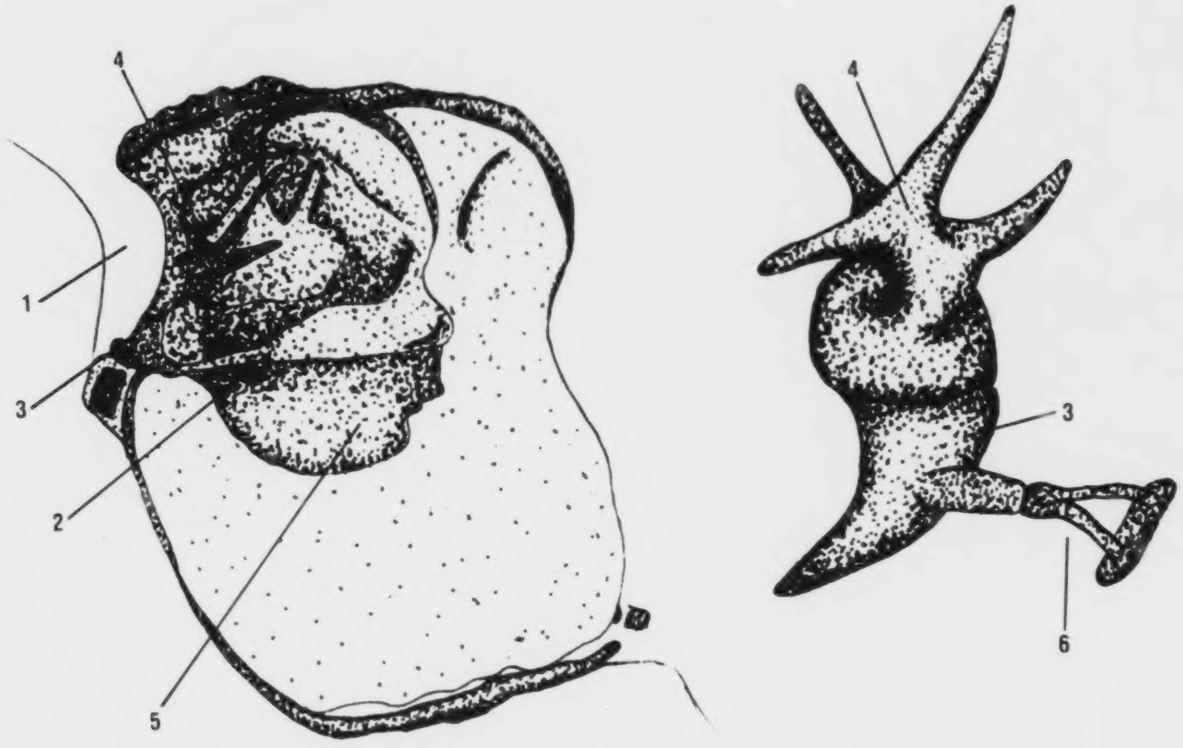
4). Lens. The lens is a spherical clear body in the center of the eyeball. The lens is attached to the ciliary body by a suspensory ligament. The lens is separated from the ciliary body by a space called the posterior chamber, and from the cornea of the eye by an anterior chamber bounded by the iris and pupil. If the vitreous body fell out of your eye, the lens might well have fallen with it. Find the lens and wash it clean of vitreous humor. You can use the lens of the eye like a magnifying glass. Place the lens over this printed page and see how it magnifies.

49. How many times does the lens magnify the print on this page? ___________

5). Pupil. The pupil is the opening through the center of the iris. Cats can see very well in the dark, in part because of the way in which their pupils are constructed. In the cat, the pupil is a vertical slit and dilates to a large, round opening in the dark. Such a large opening is not possible in a round pupil, such as that found in humans.

E. Study the cat's ear. The external ear consists of the pinnae (flaps) and a canal. The canal is called the external auditory meatus, and it leads to the eardrum, or tympanum. Refer to your drawing completed during hour one.

F. Cut away the external ear and eardrum to reach the middle ear. You should see two round, window-like holes covered by thin membranes. These are the fenestra rotunda and the fenestra ovalis. The fenestra rotunda is found in the dorsal wall of the middle ear, and the fenestra ovalis is found near the stirrup bone of the middle ear. The middle ear contains three bones, the malleus (hammer), incus (anvil), and the stapes (stirrup). The eustachian tube opens the middle ear to the nasopharynx cavity. Refer to figure 20 for aid in ear structure identification.



1. External auditory meatus
2. Fenestra rotunda
3. Incus
4. Malleus
5. Petrous bone
6. Stapes

Figure 20

The inner ear is very small and difficult to dissect. The instructor and/or students may wish, as a demonstration or optional activity, to remove the petrous bone containing the inner ear, and locate the following structures: the cochlea, utricle, saccule, and the semicircular canals.

Procedure: Hour 8

Students need the following supplies: cat, dissecting pan, probe, forceps, and dissecting scissors.

During this laboratory hour you will study the skeleton of a cat.

A. Remove the remaining flesh from your specimen. The fleshing of an animal is critical to preparing a skeleton. Take care not to cut through any remaining ligaments which hold the bones in position.

If you were to prepare a skeleton for permanent mounting, you would select a fresh, mature cat whose carcass had not been dissected. Your specimen, at a minimum, has a cracked cranium and broken ribs.

B. The bones are connected by joints or articulations. Joints allow bones to be moved in one, two, or three directions. There are six joint types. They are spheroidal (ball and socket), ellipsoidal and sellar (saddle-shaped), ginglymus (hinge), trocoid (pivot), and arthroidal (plane) joints. Study the joints connecting the bones of your specimen.

50. Identify the joint types in the joints listed in figure twenty-one below. Also state whether they can be moved in one, two, or three planes or directions.

Joint	Type	Planes Of Movement
Hip		
Knee		
Wrist		
Shoulder		
Humerus & Ulna		
Radius & Ulna		

Figure 21

C. Study figure twenty-two below, a lateral view of a cat skeleton, and find on your specimen the listed bones.

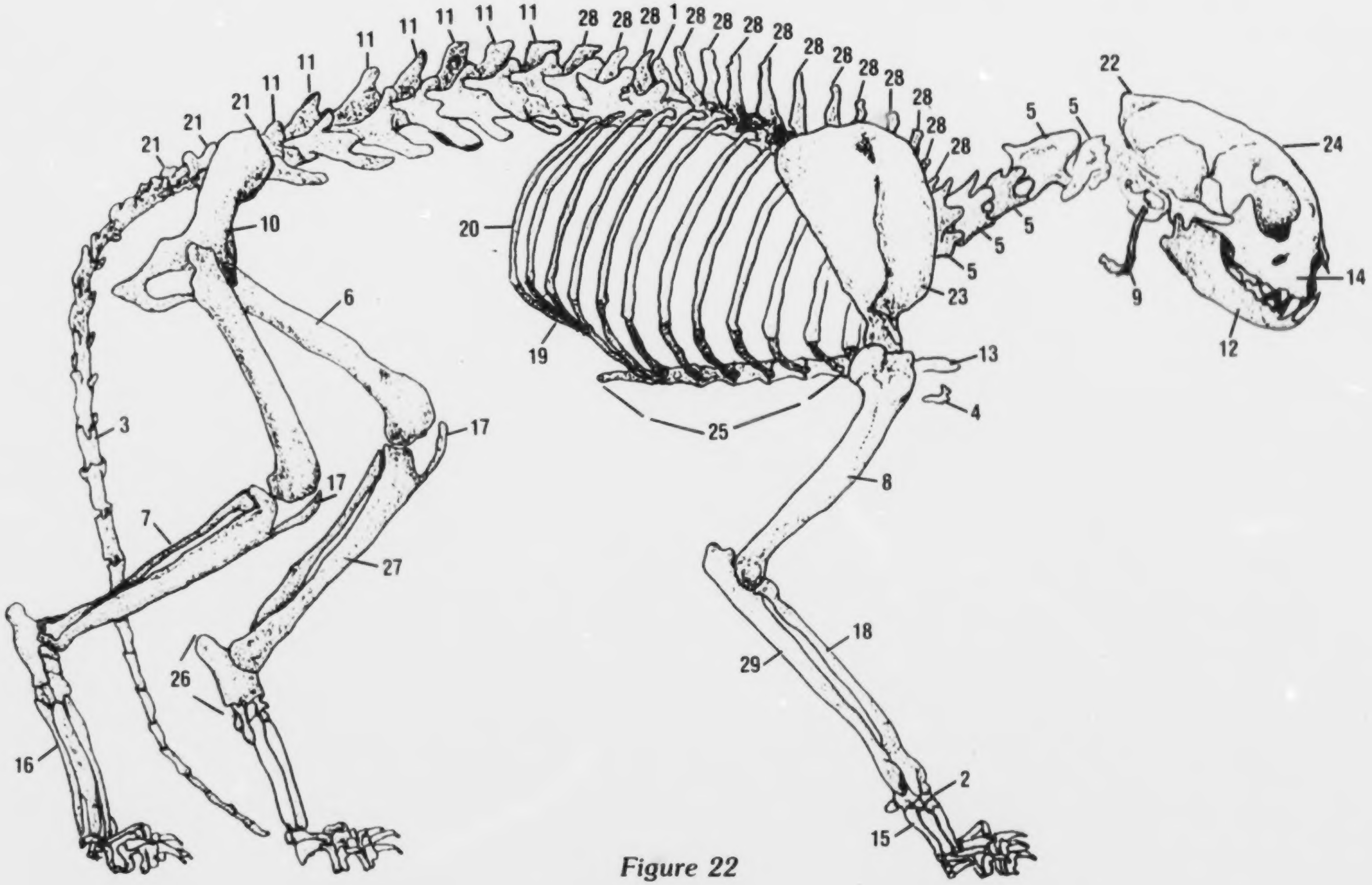

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Figure 22

1. **Anticlinal vertebra**
2. **Carpals**
3. **Caudal vertebrae**
4. **Clavicle**
5. **Cervical vertebrae**
6. **Femur**
7. **Fibula**
8. **Humerus**
9. **Hyoid**
10. **Innominate**
11. **Lumbar vertebrae**
12. **Mandible**
13. **Manubrium**
14. **Maxilla**
15. **Metacarpals**
16. **Metatarsals**
17. **Patella**
18. **Radius**
19. **Rib cartilages**
20. **Ribs**
21. **Sacrum**
22. **Sagittal crest**
23. **Scapula**
24. **Skull**
25. **Sternum**
26. **Tarsals**
27. **Tibia**
28. **Thoracic vertebrae**
29. **Ulna**

51. Identify, locate, and state the function of the bones listed in figure twenty-three below.

Bone	Location	Function
Radius		
Ulna		
Skull		
Ribs		
Tibia		
Humerus		
Sternum		
Vertebrae		
Patella		
Femur		
Tarsals		
Carpals		
Scapula		
Innominate		
Fibula		

Figure 23

Bones are for protection and/or support. The skeleton of mammals is considered to consist of two sections, the axial and the appendicular. The axial skeleton consists of the skull, facial bones, vertebral column, ribs, and sternum. The appendicular skeleton is composed of the shoulder and hip girdles and their appendages.

D. Study the bones of the head. Locate the following bones.

1). Cranium. The cranium encloses the brain. You had to crack it in order to view the brain during laboratory hour seven. The cranium consists of several fused bone structures. The frontal bones cover the area from the cat's eye (zygomatic arch) and have processes (knobs that stick out). Parietal bones cover the back section of the skull, and end in a sagittal crest. The occipital bone covers the most caudal area, and it is attached to the spinal vertebrae. The temporal bones cover the side of the skull to the ear area.

52. In the space provided below, draw the skull of your cat and label the bones that comprise it.

2). Facial bones. The facial bones consist of the nasals (paired bones roofing the ethmoid process of the occipital skull region), the palatines (forming the hard palate of the mouth), the mandible or jawbone, the teeth and the bones that contain them, the bones of the ear, and the lacrimals (small paired bones which border the lacrimal gland near the eye).

53. In the space provided below draw a frontal view of your cat's skull, and label the visible facial bones.

Optional Dissection Activity

The entire articulated skeleton of a cat may be constructed and mounted according to the following procedures. The instructor may wish to demonstrate these procedures, or students may wish to proceed with them, submitting their skeletons to the instructor for evaluation.

A. Examine the fleshed bones of the specimen to be sure that most of the natural cartilage and ligaments are intact. Place the bones in a large container and cover them with water. The container must be stone, earthenware, glass, or plastic. Do not use a metal or wooden container. Keep the container in a warm room or incubator where an average temperature of 25 to 28° C. is maintained for six to eight days. The remaining flesh on the bones will rot, or macerate. The process is complete when the flesh gels and sloughs off the bones easily, but the ligaments holding the skeleton together are still intact.

B. Remove the bones from their maceration bath, and rinse under running water, scrubbing them with a toothbrush to remove all remaining pieces of flesh. To halt any final maceration, place the scrubbed bones in a weak caustic soda solution (9 grams caustic soda per liter of water) for one to two hours. Wash the bones in running water.

C. Run a stiff pointed wire through the specimen's vertebrae, forcing the point well into the sacrum. Bend the vertebral column to its natural shape. Space the ribs equally along the vertebral column by stringing them with thread. Hold the limbs together with wire and a cork or wood support. Wire any other loose bones together in proper position. Allow the skeleton to dry for two weeks.

D. The bones will still be greasy after drying. Place the skeleton in a TF solvent bath for six to eight hours. TF solvent, or trichlorotrifluoroethane (TTE), costs more than carbon tetrachloride, but it is very much safer for students to use. The alternative is to soak the skeleton in carbon tetrachloride for the same period of time.

E. Remove the skeleton from the solvent bath and plunge it into a hot (but not boiling) water/ammonia bath for five to ten minutes. The water/ammonia bath solution should contain 0.5 liters ammonia solution in every three liters water. If it is desired to have a perfectly white skeleton, remove the bones from the water bath and place them in a ten per cent solution of hydrogen peroxide for four hours.

F. Mount the specimen using stiff wires and metal rods and attaching the specimen to a bottom board. Mounting or putting together any model is largely a matter of experience and skill, and time and patience must be applied. If you wish, a glass case may also be constructed to protect the specimen from dust and careless handling.

Resources

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Hubel, David H. "The Brain."

Iverson, Leslie L. "The Chemistry of the Brain."

Nauta, Walle J. A. and Michael Feirtag. "The Organization of the Brain."

Stevens, Charles F. "The Neuron."

Terminology

- action
- aponeuroses
- appendicular
- axial
- coelom
- fascia
- insertion
- mesentery
- origin
- pericardial
- peritoneal
- pleural
- tendon

Review

25. Cat Dissection

Name ________________________________

Date ________________________________

Multiple choice

______ 1. What are pinnae?
a. tongue c. ears
b. eyes d. toes

______ 2. The larynx is the
a. windpipe c. tail
b. throat d. voicebox

______ 3. This structure is part of the intestine.
a. ileum c. rugii
b. diverticulum d. esophagus

______ 4. The sternohyoideus is found near the
a. head c. belly
b. tail d. back

______ 5. After the soft palate is removed, you can see the
a. esophagus c. frenulum linguae
b. nasopharynx d. glottis

______ 6. The mouth, esophagus, stomach, and small and large intestines are found in the
a. isthmus of fauces c. alimentary canal
b. eustachian meatus d. duodenum

______ 7. The muscle responsible for chewing is called the
a. splenius c. masseter
b. brachioradialis d. pectoralis profundus

______ 8. The holding sac for bile is the organ
a. liver c. kidneys
b. pancreas d. gall bladder

______ 9. Which set of bones connect the phlanges to the tarsals?
a. metacarpals c. metamarsals
b. metatarsals d. metaphlanges

______ 10. The pancreas secretes
a. insulin c. gonadotropin
b. thyroxine d. FSH

______ 11. The diaphragm is a
a. muscle c. stomach section
b. intestine d. umbilical cord

______ 12. Molars are
a. toes c. fingers
b. tongue d. teeth

______ 13. A vestibule is found
a. in the bile duct
b. between teeth and lips
c. in the nasopharynx
d. only in a cat

______ 14. The adrenals sit on top of the
a. kidneys
b. liver
c. gall bladder
d. heart

______ 15. The brachiocephalic's insertion is at the
a. distil end of the humerus
b. distil end of the femur
c. anterior deep pectoral
d. aponeuroses

______ 16. Surrounding the walls of the thoracic region is the
a. humerus
b. latissimus dorsi
c. shin
d. gluteus maximus

______ 17. The tricuspid valve is found in
a. all arteries
b. heart
c. pulmonary veins only
d. brain

______ 18. The glottis lies between the
a. trachea and larynx
b. alveoli and bronchioles
c. bronchioles and bronchii
d. nasopharynx and larynx

______ 19. The choroid is found in the
a. heart
b. tail
c. brain
d. eye

______ 20. Cornu are found in what cat system?
a. respiratory
b. reproductive
c. digestive
c. circulatory

Matching: entries in the second column can be reused.

______ 21. pleural
______ 22. appendicular
______ 23. fascia
______ 24. insertion
______ 25. pericardial
______ 26. axial
______ 27. intestinal
______ 28. ovarian
______ 29. urethra
______ 30. parietal

a. digestive
b. circulatory
c. respiratory
d. excretory
e. reproductive
f. muscular
g. skeletal
h. nervous

Answers Found: p 305 - #1 & 12; p 308 - #16, 23, & 24; p 310 - #4; p 311 - #15; p 312 - #7; p 315 - #11 & 25; p 316 - #8; p 317 - #10; p 319 - #14; p 320 - #6 & 13; p 321 - #2, 5, & 18; p 323 - #3; p 325 - #21; p 327 - #17; p 332 - #29; p 336 - #20 & 28; p 339 - #19; p 342 - #9; p 343 - #22 & 26; p344 - #30.

26. Laboratory

Animal Behavior

When you have completed this laboratory you should be able to:

1. Explain the theoretical difference between innate and learned behavior.
2. Define taxis and state three types of stimuli which can cause a taxic response.
3. Describe conflict behavior and explain why it is thought to be important in the development of threat displays and courtship behavior.
4. Describe how threat and appeasement behavior help maintain a dominance hierarchy.
5. Describe territorial behavior.
6. Describe social behavior and give two examples of the communication patterns involved.

Man's Best Friend

Humans often fallaciously assume that animals act because of emotions and drives similar to those of man. We tend to think, for instance, that a dog displays shame when it puts its tail between its legs or crawls on its belly after receiving discipline from its master. What we actually observe in such cases are appeasement displays innate to social mammals. The dog thereby reaffirms its acceptance of subordination in the human/dog society in which it exists. All domesticated mammals derive from social species in which hierarchial position is communicated by innate behavior. This behavior should not be confused with human emotions, rather we need to understand the animal in its own terms.

Ethology

Man has observed animals since before the dawn of written history. Prehistoric man painted animals on cave walls, the Chinese developed metaphors of animal behavior to describe human relations, and Greeks believed in gods whose half animal bodies symbolized that god's powers. The Greek philosopher Aristotle wrote a book which focused on animal behavior. Aesop told tales about animals, and he, as did his people, made inferences about human behavior from what they had observed among animals. Medieval Japanese scroll paintings and stories employed animals to satirize human foibles, as did the modern writer George Orwell in *Animal Farm*. Most of this human observing and commenting has failed to deal with the animal in terms of the animal. The focus, rather, has been upon man and how the animal impacts human society.

Ethology, a word first used shortly after 1850, applies to the study of animals under natural conditions. Europeans, such as the Austrians Konrad Lorenz and Karl von Frisch, pioneered the use of careful, systematic observations to explain animal behavior, with observations and experiments employed to test hypotheses they had developed. For their work, Konrad Lorenz, Karl von Frisch, and Niko Tinbergen, a Dutchman and student of Konrad Lorenz, won the Nobel Prize in 1973. Konrad Lorenz is known as the "father of ethology."

Many animal behaviorists have emulated Lorenz's methods of scientific field research. Jane Goodall of Great Britain, who was supported by the National Geographic Society under the aegis of Louis Leakey, spent most of her adult life studying the habits of chimpanzees. Dian Fossey, an American who was similarly funded and supported, opened our eyes to the intricate society of the mountain gorilla, before she was hacked to death by poachers.

Animal Behavior in the Laboratory

At the same time that ethology was being developed as a science by Europeans, an American school of animal psychologists pursued the goal of learning about animal behavior under controlled laboratory conditions. This behaviorist school was strongly influenced by Ivan Petrovich Pavlov, a Russian physiologist (1849-1936) whose classic studies on dog responses are still pertinent. B. Frederic Skinner of Harvard University pioneered mechanized tests of both animal and human behavior. Harry F. Harlow, director of the Primate Laboratory at the University of Wisconsin, studied parent/child bonding with newborn rhesus monkeys and artificial mother figures made of wire, baby bottles, and sometimes terrycloth.

Many scientists today employ a variety of methods in their observations on animal behavior. Donald R. Griffin, at the Tropical Research Station of the New York Zoological Society in Trinidad, theorized that bats make use of stimuli beyond the range of human sense organs, and he proved his theory by recording ultrasonic sounds bats emit to locate objects as they fly, a sonar-like mechanism. Theodore C. Schneirla, of the American Museum of Natural History in New York City, combined years of field work with laboratory experiments to become an ant expert. He discovered the phenomena of chemically-driven ant suicide, in which ants blindly follow their chemical scent, even until death if the pheronomes have been laid down in a circle.

Kjell Sandved, one of the world's leading nature photographers who works from the Smithsonian's Museum of Natural History in Washington D.C., has documented moth behavior in over thirty countries, as well as photographing natural phenomena as diverse as Bronze Age man near the Dead Sea and the wildlife of Madagascar. He has provided a visual study guide for those interested in life.

Patterns of Response

Behaviorists have articulated three response patterns which contribute to a specie's survival. First, an animal must be able to discriminate between important and unimportant stimuli in order to survive or reproduce. For example, chicken hens which lay eggs in response to longer-light days, even though not fertilized by a rooster, would not be aiding in the survival of the species, if in a natural environment (refer to laboratory twenty-four).

Second, behavior patterns must allow an animal to solve immediate problems, such as feeding when hungry, or escaping when threatened by predators. Once adaptive response patterns can become inappropriate, such as the circling behavior of Arctic musk oxen, which protects calves and cows from wolves but which also groups the bulls for easy slaughter by humans with rifles.

Third, behavior patterns will be naturally selected as they contribute over time to an organism's reproductive success. For example, Niko Tinbergen, a leading Dutch ethologist at Oxford University, has demonstrated that black-headed gulls which do not remove empty eggshells from the nest lose more chicks to predation, apparently because the solidly white interior of the speckled eggshell helps predators spot a nest with chicks. Most black-headed gulls instinctively remove eggshells shortly after hatching.

Instinctive versus Learned Behavior

Members of the same or similar species tend to show the same inherited behavior whether they are domesticated or wild. Thus, the domestic dog displays social behavior interited from wild ancestors. These types of behavior are considered to be innate, or instinctive. Learned behavior, on the other hand, results from adjustment to a new environment. To a dog, the human master substitutes for the pack's alpha animal. A barking, trick-performing seal at a show certainly has learned a new form of feeding behavior. Of course, learned behaviors can become integral, if the stimulus remains the same. For example, humans do not consciously think about the muscles required to write or ride a bicycle, but once mastered the appropriate neural sequencing remains engrained. Animals with longer life spans tend to acquire more learned behaviors.

In this laboratory you will observe animal behavior during four mini-labs, each of which is separable and expandable into lengthy experiments of many months duration. These four hours introduce some aspects of both laboratory and field research into animal behavior. You will work with fruit flies, Siamese fighting fish, chickens, and honey bees. If it is not possible to undertake one of the sessions, use those which are practical in your locality.

Pre-lab

Supplies needed:

Equipment

watches or stopwatches
Glass Y's to connect vials
3 vials with 1 holed stoppers
bee veil
petri dish
compass
light source
2 aquaria with heaters and pumps
fish net
hand mirror
brooder
chick feeder and water dispenser

Materials

Drosophila culture
Drosophila food or decaying fruit
hatching chicks
2 male Siamese fighting fish
  (Betta splendens)
bee hive with bees
adrenalin injection kit
chick feed
aluminum foil
black paper
ammonia
filter paper
masking tape
dechlorinated tap water
heavy nonwoven interfacing fabric
plastic transparent thread or
  fishing line
fabric paint, crayons, or markers

Special Preparations

1) *Drosophila* culture: fruit flies may be caught in season, or purchased from biological supply houses. If you are running this experiment in Minneapolis during the winter, for example, you had best purchase the fruit flies. To catch fruit flies, use any fermenting fruit, but ripe banana is excellent. To speed the fermenting process, dip a piece of ripe fruit in a yeast suspension made by dissolving yeast in warm water. Place a piece of paper toweling, which will absorb excess liquid, into the bottom of a milk bottle or vial. Then insert the yeast covered fruit. Leave the container open to attract flies. When the fruit flies have begun to feed on your attractant, plug the neck of the glass container with cotton wrapped in cheese cloth.

If either purchased or captured fruit flies have to be held longer than two or three days, you will need to prepare a medium which will not liquify and drown the flies (rotting banana, for example, liquifies in a few days). Prepare 50 to 60 ml. of medium for each container. Heat the medium close to boiling, and pour it into sterile vials or small milk bottles. Insert a strip of paper toweling into the medium while it is still soft, with an end of the toweling sticking out to provide surface area for egg laying and pupation. Cap the bottles, and allow the medium to solidify on a slant, to provide more surface area for feeding. These containers with medium can be prepared well in advance of fruit fly arrival, if refrigerated.

To transfer captured flies to the prepared-medium container, place the new container upside down over the unplugged trap, and fruit flies will fly up into the new container.

Medium: dissolve 15 g. agar in 480 ml. water. Stir well and heat to boiling. Add 500 g. mashed banana. Or, if bananas are unavailable, add 135 ml. corn syrup or molasses and 100 g. cornmeal dissolved in 270 ml. cold water. Gently boil the whole mixture for about five minutes. If desired, add a mold inhibitor according to package directions. The mold inhibitor is optional, but recommended for hot, humid climates. Mold inhibitors, such as Tegosept M (methyl paraben), are available from biological supply houses.

2) Chickens or other fowl: **for imprinting to be successful, chicks newly emerged from their shell must be used. This laboratory hour must take precedence over any other activity. It must run as the chicks hatch** (see laboratory twenty-four). Most laboratories can be re-scheduled or interrupted, but not this one.

3) Male Siamese fighting fish must be kept in separate aquaria or compartments. All visual contact between individual fish should be blocked until the experiment. They may be purchased from tropical fish stores or biological supply houses. Water temperature of all aquaria or containers should be similar (25° C.) so that the fish do not suffer temperature shock during introduction. Also, prepare the water by aeration for a day, or use a dechlorinating agent, before introducing the fish. If the aquaria or jars are tiny, loosely cover them so that the fish will not jump out. Air pumps are required to keep the fish any length of time, but they are not essential in the short term of one week or less. Siamese fighting fish are able to gulp air from the water's surface and store the air in a pair of internal chambers above the gills. Filled with thin plates covered with fine blood vessels, these auxillary breathing organs provide for a direct exchange of oxygen and waste gases.

During the laboratory fighting fish models will be made, unless you wish to prepare them in advance. Use any waterproof, stiff material. Thick plastic is ideal but not always available. Fabric stores regularly stock heavy, nonwoven interfacing, a washable material used to stiffen collars and form flannel board figures. When colored with permanent fabric markers, they may be cleaned and reused for many years. Each student group may wish to make their own, or the instructor may wish to provide uniform fighting fish models for the class as a whole. The models will be lowered into the aquaria by transparent line during the laboratory.

4) Bees: bees and hives are available from biological supply houses, mail-order houses, and local beekeepers. Some local beekeepers are happy to care for hives in exchange for their honey harvest. If purchase and maintenance of a hive are not practical, temporarily borrow a beehive, locating a willing lender through the local apiary society. Beekeepers usually are delighted with interest shown in their avocation and are excellent sources of practical information.

Bee veils are not essential for the type of hive observation to be performed during the laboratory, but they can comfort the timid student or inexperienced instructor. Careless approach or careless observation of the hive might lead to a bee sting. Before students get near the hive, check to see if any of them have a known allergy to bee stings. In any event, have an injection of adrenalin available in case a stung student suffers a generalized allergic reaction. Effective epinephrine injection kits are available from pharmacies and doctors. If refrigerated, epinephirine injection kits are viable for up to one year.

Observation bee hives are fascinating additions to biology programs. They provide a long-term source of interest for very little effort.

Time Required

The animal behavior laboratory requires four classroom hours, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the following: *Drosophila* culture, food vials and stoppers, glass Y's to fit the vials with stoppers, rotten fruit, ammonia, black paper, aluminum foil, filter paper, watch, light source, and masking tape.

During this laboratory hour you will study taxis in fruit flies. Taxis is any orientation behavior in which there is a sustained movement either toward or away from a stimulus. Taxic responses are termed phototaxic if the stimulus is light, geotaxic if the stimulus is gravity, and chemotaxic if the stimulus is chemical in nature.

Steps

A. Put five or seven flies in a clean vial (this move can be a challenge to the less well coordinated). Cover the opening with another vial of the same size, and tape the vial openings together so that the flies can move freely in both vials. Cover the taped vials with black paper so that only one of the ends opens to the light, and lay it down **horizontally**. Place a light source near the open end of the vials, and wait three minutes. **Quickly and with as little disturbance as possible,** remove the paper and count the number of flies in each section of the tube.

1. How many flies were in the section closest to the light source?

____

2. How many flies were in the section farthest from the light source?

____

B. Repeat the experiment twice more.

3. Record your results in the chart, figure one, below.

Trial	Near Light Source	Near Closed End
1		
2		
3		

Figure 1

4. Do fruit flies show positive or negative phototaxis? ________________________

____

5. Why was it important to keep the vials in a horizontal position during the experiment?

____

____

C. Temporarily remove the black paper and allow the five to seven flies inside the taped vials time to alight on the glass and come to rest. Once at rest cover the vials completely with black paper. Hold the vials **vertically** for three minutes. **Quickly and with as little disturbance as possible,** remove the paper and count the number of flies in each section of the vial tube.

6. How many flies were in the top section? ________________________

7. How many flies were in the bottom section? ________________________

D. Repeat the experiment twice more, reversing top and bottom sections.

8. Record your results in the chart, figure two, below.

Trial	Top Vial	Bottom Vial
1		
2		
3		

Figure 2

9. Do fruit flies show positive or negative geotaxis?

10. Why reverse top and bottom positions of the taped vials?

11. What did your tests reveal regarding the relative strength of response to phototaxis versus geotaxis in fruit flies?

12. Devise an experiment to test phototaxic and geotaxis strength in fruit flies. Explain how you would do this in the space provided below.

E. Set up a T maze using three vials and stoppers and the Y glass to join them, according to figure three on the following page. Place a small piece of fermenting fruit smeared on a small square of filter paper in one vial before attaching it to the T maze, nine to thirteen flies in another vial before attachment, and a small square of moistened filter paper in the third vial. The third vial will function as a control. Watch the flies for five minutes.

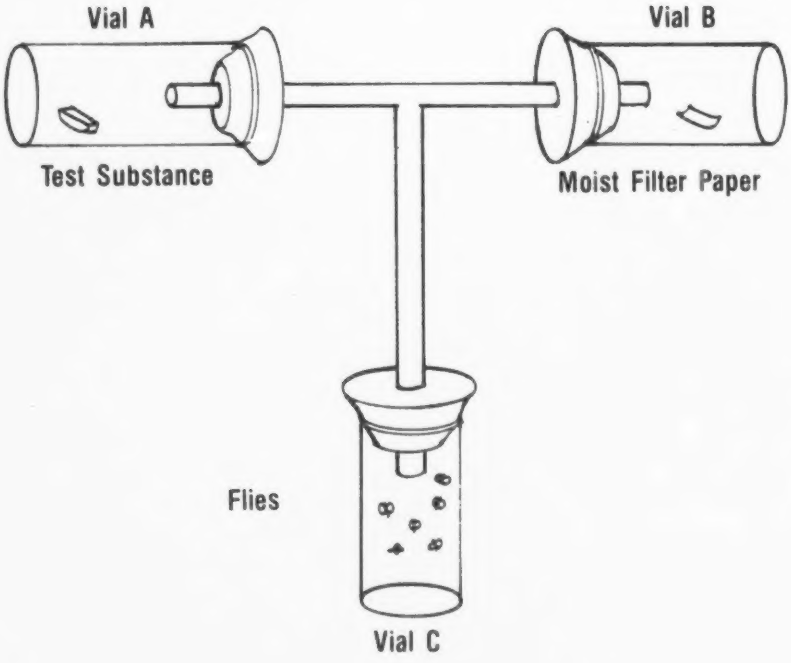


Figure 3

13. After five minutes, how many flies are in the original vial section? ____________

14. How many flies are in the food vial section? ____________

15. How many flies are in the moistened filter paper section? ____________

F. Wait five minutes more.

16. Are the numbers of flies in each vial section the same? If not, how have they changed? ____________

____

____

G. Repeat the experiment once more, but substitute an ammonia soaked paper for the fermenting fruit pulp paper.

17. Record your results with both food and ammonia in the chart, figure four, below.

Trial	**Substance Vial**		**Water Vial**		**Fly Vial**	
Minutes	**5**	**10**	**5**	**10**	**5**	**10**
1 Food						
2 Ammonia						

Figure 4

18. Do fruit flies show positive or negative chemotaxis towards fermenting fruit? ____________________

19. Do fruit flies show positive or negative chemotaxis towards ammonia? ____________________

20. What might the biological advantage be to the fruit flies for these different responses? ____________________

____

____

Procedure: Hour 2

Students need the following: hatching chickens (or other hatching fowl), and brooder with food and water trays.

During this laboratory period you will observe chicks (or other fowl) hatching, and you will attempt to imprint yourself as a chick surrogate parent. **Step A must be completed prior to the imprinting laboratory hour. Step B is best accomplished when the chick is between ten and sixteen hours old.**

Imprinting

Imprinting, the rapid, early learning of behavior appropriate to the species, has been observed in insects, fish, birds, and mammals, although it has been studied most extensively in birds. Human infant bonding patterns and earliest behavior are undergoing extensive research, and behavior similar to that observed in birds has been noted. Imprinting became widely known as a result of the work of Konrad Lorenz, who first used the term technically in 1937. Lorenz is perhaps best known for demonstrating that goslings follow the first moving object seen mmediately after hatching.

Lorenz also discovered that there is a relationship between imprinting and sexual behavior in male birds. For example, a male Chinese pheasant will provide a courtship display to objects similar to that to which he was imprinted as a chick. Lorenz imprinted himself as parent to a male jackdaw, and when the bird achieved sexual maturity, the jackdaw kept trying to stuff worms in Lorenz's ear in a misplaced effort at courtship. This occurred before Lorenz had made the connection between imprinting and adult sexual behavior.

Some birds are easier to imprint than others, and the instinct seems to be genetically conditioned. Experiments have been run variously on chickens, ducks, and geese, with the more easily imprintable of the species separated from the more difficult, and the separate groups then inbred. The offspring displayed imprinting capability very close to that of the parents.

Steps

A. **This first step in the procedure likely will not take place during a scheduled laboratory period.** Whenever hatching begins in a clutch of eggs, students should observe further hatching through the incubator's window or plastic cover, without removing the lid. Hatching cannot be orchestrated, and whenever it occurs, students will have to make themselves available. Do not remove the lid of the incubator because both hatched and unhatched chicks are very susceptible to chilling during this period. Chicks can live 48 hours before requiring food and water, so do not attempt immediately to feed them.

21. What kinds of movements do the chicks employ in getting out of the shell? ____________________

____

____

22. Study the empty shell. Draw the pieces in the space provided below.

23. Describe the pattern in which the egg was cracked

24. Do you think all bird's eggshells are cracked in the same manner? Why or why not?

25. Can you cite any evidence for your statement? If so, what?

B. As some students will have to crawl on the floor, they should come to class in casual dress, or perhaps use a large, washable throw rug or blanket. Students need to work in groups of at least two, because while one student attempts to become the chick's surrogate parent, another student must record the chick's actions and reactions.

Imprinting behavior is strongest in chicks ten to sixteen hours old. Select the chick you will attempt to imprint. The surrogate parent should gently pick up the chick from the incubator, and cuddle it in the hands. Softly and rhythmically talk to the chick, repeating over and over the name you have decided to call it. Slowly move to your prepared work area of the room.

C. Place the chick on a soft floor surface, keeping your hands cuddled around it. Continue to call softly to it. After a few minutes, move your cupped hands twenty to thirty centimeters away. Coax the chick to walk towards your hands by calling to it and moving your hands slowly back and forth. When the chick takes a step or two in the direction of your hands, reward it by cuddling it some more. Set it down and repeat the procedure. Keep moving your hands farther and farther away each time you repeat the procedure. **Be patient.** Hand imprinting should take ten or fifteen minutes to accomplish.

26. In the space provided below, record what the chick does during hand imprinting. Record whether or not it stands, sits, or makes random or purposeful movements, such as those toward or away from the surrogate parent. Record these movements minute by minute.

D. When the chick has been trained to come to your hands, it then needs to be trained to focus on another part of your body. People are very large compared to chicks, and the chick must learn to associate your feet with the parent surrogate, as well as your hands, so that the chick can be trained to follow you.

Put the chick down by your heel, and take one small step while calling to it. Wait until it catches up to you, then take another step. Gradually increase the speed and length of your steps, making sure the chick can keep up. Continue softly calling as you walk. Eventually the chick should follow you wherever you go.

27. In the space provided below, record what the chick does during heel imprinting. Record whether or not it stands, sits, or makes random or purposeful movements, such as those toward or away from the surrogate parent. Record these movements minute by minute.

28. Compare your chick imprinting observations with those of other students. Did the chicks all show similar types of movements? ____________ Comment: ____

29. How many times was each chick cuddled? How long did hand imprinting take in each group? Answer these questions in the chart, figure five.

Group	Times Cuddled	Imprinting Time
Your Group		

Figure 5

Optional Activities

Students may wish to explore some variables concerning imprinting and chick behavior. They can submit their results to the instructor in a formal laboratory report.

1) Formally observe the imprinted chick with its surrogate parent for at least one week. Does it continue to follow the surrogate parent? What sorts of behaviors on the part of the surrogate parent tend to reinforce imprinting behavior? Compare your findings with other student groups.

2) After completing procedures in this laboratory, try to imprint older or younger chicks. Does there appear to be a critical period during which imprinting occurs? Compare imprinting success with chick age.

3) At about two weeks of age chicks no longer need artificial heat, over and above room temperature. At that time provide a number of them with a box to live in, a penned enclosure, and food and water. Mark each of them on the back with a different color, so that you can tell them apart. (Fat magic markers work well). Formally observe chick behavior at the same time for the same period each day, and record your observations. Can you determine a pecking order? How did it evolve? Compare chick growth and development with their position in the pecking order.

4) You can also run preference tests on the chicks. What are their color preferences? Make small construction paper boxes of different colors and design an experiment to test which box gets pecked at most often, and the order of pecks each colored box receives. Change the order of the boxes. Does the position of the colored box affect its pecking order?

5) Vary the above experiment by making different shaped objects, such as rectangles, squares, triangles, hexagonals, etc., all of the same color. Determine which shape gets pecked most often and the packing order of the shapes. Does the position of the shape affect its pecking preferability?

6) Raise a chick at home. When it is an adult, introduce it to a flock of chickens. Observe its behavior. Does it relate well to other chickens, or does it show identity confusion? What do you observe? How long does it take to integrate into the flock? What is its position in the flock pecking order? Compare its adult behavior to that of other chickens in the flock.

7) Attempt to imprint yourself as a surrogate parent to a mammal. This has been successfully accomplished with guinea pigs.

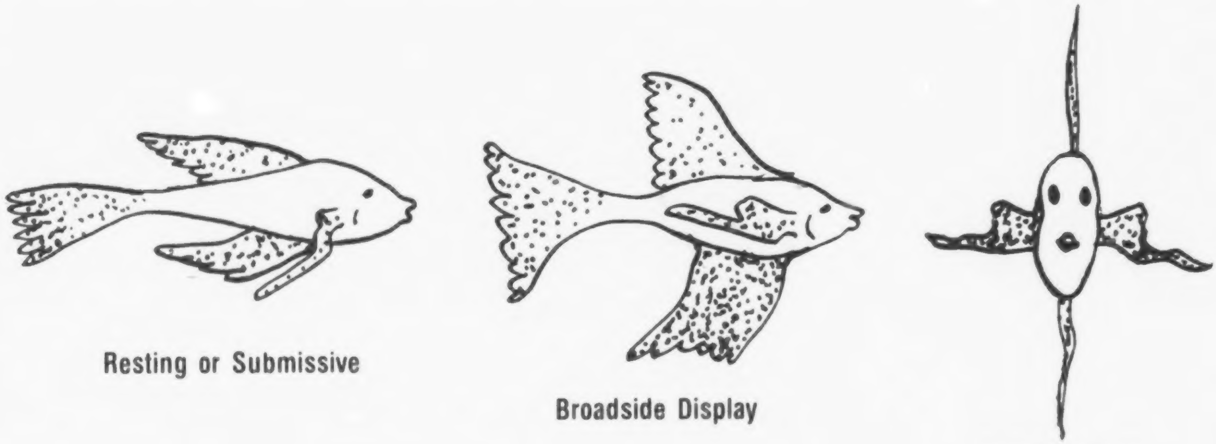
Procedure: Hour 3

Students need the following: two male Siamese fighting fish, two small aquaria with heaters and pumps, hand mirror, plastic transparent thread or fine line, fabric markers, scissors, and heavy, nonwoven interfacing fabric.

During this laboratory hour you will observe fish bred for exaggerated conflict, or agonistic display. Agonistic behavior in these fish is stimulated by protective territorial instincts. Protection of offspring, territoriality, and courtship, all are expressed in conflict.

Siamese Fighting Fish

Siamese fighting fish, *Betta splendens*, belong to the Asiatic family *Anabantidae*. They live naturally in the tropical freshwater streams of Southeast Asia. Fish available for purchase have been bred not only for their beautiful colors, most notably the tail, but also for exaggerated conflict behavior displays. The male fish shows its intention to attack another male, or to flee, by visual dispays. Normally the fish is as drab as the female, or only slightly colored, and it keeps its dorsal and ventral fins folded. During displays fighting fish often become brilliantly iridescent. Some of these displays are illustrated in figure six.



Resting or Submissive

Broadside Display

Frontal Attack

Figure 6

One of the male fish will eventually intimidate the other and bluff its competitor into leaving the territory. When the fish are housed in aquaria, the intimidated fish unfortunately cannot leave, and the winner will chase and bite the losing fish. **In your experiment, you must be ready to remove one of the fish as soon as the winner is apparent.**

The decision to fight or flee is strongly dependent on whether it is in its own established territory, and whether it has just defeated another fish or has just been defeated itself. The most impressive displays are observed with closely matched fish.

Nest Defenders

Some fish which have not evolved other means to guarantee survival of the next generation achieve this survival by protecting nest and territory. Male Siamese fighting fish are well known for guarding their nests with ferocity. This ferocity can be misplaced, but the initial behavior is one of protection.

During courtship the male chases, nips and butts, and eventually reduces the female to submissiveness. Male Siamese fighting fish maintain visual display during mating, and the female must make herself as inconspicuous as possible to avoid being attacked. The male wraps itself around the female and appears to squeeze the eggs out of her body. The male fertilizes the sinking eggs, and then grabs the eggs in its mouth and fetches them to a floating mucous bubble nest it has previously built. The male then guards the eggs against all territorial invaders, including the mother. It will continue this vigil until the hatched fry are six days old.

This ferocious defense of territory and young can be stimulated in response to any territorial invasion, by other Siamese fighting fish or by artificial stimuli. Artificial stimuli, such as models or brightly colored objects, are most effective if the fish has recently defeated another fish and is in its own territory with the motivation to display high.

Steps

A. Place a male fighting fish in an aquarium, and observe its normal behavior. Place a second male fighting fish in the aquarium, and observe the behavior of both. Note their orientation to each other, their color, the positions of their fins, and the position of their gill covers, or opercula. Continue your observations for ten minutes, or until one of the fish starts pursuing the other. **If this happens, immediately remove the aggressor fish.**

30. Record what you observe in the space provided below

31. If the fish were equally matched before the experiment, which one theoretically has the advantage? Why?

B. Substitute for the removed dominant fish one which was submissive from another group (groups will have to double-up). Note their orientation to each other, their color, the positions of their fins, and the position of their gill covers, or opercula. Continue your observations for ten minutes, or until one of the fish starts pursuing the other. **If this happens, immediately remove the submissive (non-attacking) fish.**

32. Record your observations in the space provided below.

33. Did either of the submissive fish show conflict behavior? If so, which one? Why?

C. Place a dominant fish in an aquarium, and allow it five minutes to adjust to the new territory. Hold a mirror alongside the tank close to the fish, and move it slightly so that the image appears to move. **Be patient.** Continue for five minutes, or until there is a response, whichever occurs first.

23. What kinds of behavior did the fish display toward its mirror image? ______________________________

____

____

____

D. Make two models of the dominant fish using nonwoven interfacing material (or heavy plastic) and scissors. Cut one model out in the shape of the fish when it is in full broadside display (see figure six). Cut one model out in the shape of the fish at rest (also figure six). Color the models in a fashion similar to your dominant fish. Tie transparent thread or fishing line to the models and wet them.

E. Lower one of the models into the water near the fish. If the fish moves away, follow it, but do not touch the fish. Continue to present the model for five minutes, or until there is a response, whichever occurs first.

F. Repeat step E using the other model made during step D.

35. Record your observations for steps E and F in the figure seven chart.

Reaction	Broadside Display	Rest
No response		
Swims away		
Lowers ventral fin		
Raises dorsal fin		
Tail expands		
Color increases		
Operculae extend		
Faces model		
Bites model		

Figure 7

36. Was the response greater or less than the response to another fish? Why? ______________________

____

____

Optional Activities

Students may wish to explore some variables concerning fish behavior. They can submit their results to the instructor in a formal laboratory report.

1) Repeat steps D. E. and F. keeping the shape of the object the same but varying the color. Did color or shape more strongly influence the response of the fish?

2) Consider other fish and their behavior. For example, schooling is prevalent among many fish. It is a form of social communication. What causes fish to school? Make nonwoven interfacing models similar to the fish in an aquarium. The aquarium may be stocked with groups of schooling fish, such as zebra danios, white clouds, or neon tetras. Devise an experiment to determine what types of artificial visual stimuli, such as shape or color, are required to cause fish to group around and follow the stimulus. Do fish without obvious visual markings react similarly to fish with obvious visual markings?

Procedure: Hour 4

Students need the following supplies: bees in a beehive, compass, magic markers, and an optional bee veil. Additionally, the instructor should have available an adrenalin injection kit, in case of an accidental bee sting to which some observer may be allergic.

In this laboratory hour you will observe a colony of social insects, bees, and their interactions. You also will diagram bee dances and observe the relationship of these dances to locating foods sources.

Honey Bees

Honey bees are social insects with a very high degree of specialization. The female worker bees are sterile, and they leave all egg laying to the queen. The workers exist to benefit the colony as a whole. Working themselves to death within a few weeks during the height of summer, they tend the queen, brood cells, and the hive, and when older begin to forage away from the hive for pollen and nectar. Food gathering is a communal affair, and information on its location is exchanged by a successful forager in a variety of elaborate ways.

A honey bee colony may number as many as 80,000 individuals, and it consists of a fertile diploid female called the queen, workers which are infertile diploid females constituting more than ninety five percent of the hive's numbers, and drones which are haploid males. The drones do nothing but wait for an opportunity to fertilize a virgin queen during her nuptial flight, thus contributing a colloquial word which describes a non-productive member of human society. This degree of specialization is similar in many other social insects, such as ants and wasps, in the order *Hymenoptera*.

Queens, Drones, and Workers

Bee bodies are divided into three parts, head, thorax and abdomen. The head has compound eyes. These eyes are larger in the drone than in the worker or queen. The head also has short, thick, smooth mandibles, which work sideways, instead of up and down as in higher animals. The mandibles have no teeth, but they do allow hive bees to conduct necessary hive duties such as molding wax into comb. Bees also have a tongue and three pairs of salivary glands, the largest pair of which is used to make food for larvae. The antennae, or feelers, are two long, horn-like structures which protrude from the head of the bee. The feelers appear to serve the purposes of smell, touch, and hearing.

The honey bee has four wings and six legs fastened to the thorax. In flight each of the two sections of wing on either side of the body link together with fine hooks to increase flight surface area. In the hive the wings are folded back on each other to expose a minimum of surface area. The legs have claws, and also a pocket which secretes a sticky substance. This sticky substance enables bees, like flies, to walk on smooth surfaces. The anterior legs also have a notch and a thumb-like spine which is used to clean the antennae. The third pair of legs, in worker bees only, has a hollow portion, called a pollen basket, which enables the bees to carry pollen, a protein source, back to the hive.

Breathing organs are in the thorax and in the abdomen between the rings or segments of the third body section. The first stomach, or honey sac, is located in the abdomen. The bee can digest honey and nectar by forcing it into a second stomach for digestion. Or, it can regurgitate the contents of the first stomach into hive storage cells where fresh nectar is concentrated through evaporation (aided by hive bees beating their wings) and altered by enzymes to become honey. In a way, honey can be regarded as a form of bee vomit.

Ovaries, or egg pouches, are very large in the queen, and incomplete in worker bees. Drones do not have stingers. Queens have stingers, at the end of the abdomen, which are curved and used as an ovipositer and in fights with other queens. Queens can sting many times. Workers, on the other hand, have a straight stinger which is barbed and attached to a poison sac. Worker bees use the stinger once. Their sting usually is suicidal, in that the barbed stinger and poison sac are firmly attached to the intestinal tract. Workers with filled stomachs, such as a returning field bee or swarming bees which have filled their honey sacs before leaving the hive, have distended abdomens and cannot bend to sting until they dump their honey load.

Study figure eight which diagrams the three types of honey bee, so that you will be able to identify them in field or hive.

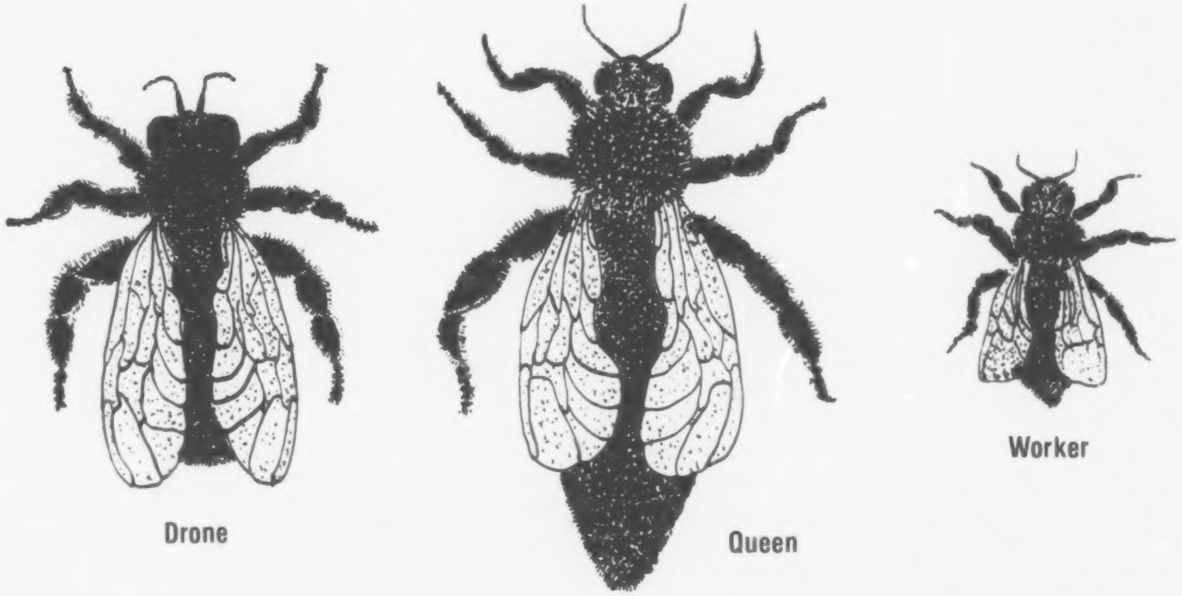


Figure 8

Reproduction

Reproduction begins with the mating flight of a virgin queen. She receives enough sperm during this one mating to last the rest of her egg-laying life, although she may mate with more than one drone. The drones are killed by the act of coupling. Mating takes place when the queen is five to six days old and occurs on the wing. If mating is delayed beyond two weeks the queen loses the instinct to do so, and she begins laying infertile eggs which will all be haploid drones (desperate worker bees in a queenless hive also begin to lay eggs, but all these too will be drones). The newly fertilized queen will function as an egg-laying machine for the balance of her life of perhaps five to seven years. Life span is greatly dependent upon her effectiveness as an egg-laying machine. The workers replace a queen whose output begins to drop. Beekeepers also change hive queens every two to three years for peak hive efficiency, for a young, healthy queen can lay as many as three thousand eggs a day.

The queen uses the sperm received during her mating flight to fertilize eggs as she lays them one to a cell. Cells are prepared and eggs attended by "nursery bees." The egg develops first into a larva and then into a pupa. Eggs in cells selected to produce queens are fed a pharyngeal proteinaceous excretion called royal jelly during their entire development, whereas worker bees and drones are fed royal jelly only during the first three or four days of development. During the remainder of their time as larvae, workers receive a coarser food, which is a mixture of pollen and honey called beebread. Groups of young worker bees care for the young, feeding each of them two or three thousand times a day during the six or so days of their larval life stage. Workers cap the cells with wax during pupation. Calculations show that one worker bee working full time can tend only two to three larvae at a time. Queens emerge from the pupa in fifteen days, workers in twenty one days, and drones in twenty four days.

Not all of the queen's eggs are fertilized; those that are not develop as haploid drones who are 100% genetically related to the queen and to each other. The diploid queen, however, is only 50% related to her sons, because she only donated 50% of her genes to the drones. Workers share the same haploid father, whose sperm is genetically identical, and therefore are 100% related on the father's side. Workers share the same diploid mother, whose eggs have a 50% chance of relatedness. Therefore, workers share a 50% relatedness with the queen, but a 75% relatedness with each other. Workers have a better chance of reproducing themselves by protecting the queen than they do by reproducing themselves.

Worker Life Stages

When a worker bee first emerges from the pupa, she busies herself cleaning out newly vacated cells to prepare them for a new generation of larvae. After two or three days, her salivary glands begin to secrete royal jelly and she feeds very young larvae. When larvae are older, she weans then from royal jelly by feeding them beebread. At the age of seven or eight days the young worker bee will begin short orientation flights from the hive.

After the tenth day the secretion of royal jelly stops, and the wax glands begin to function. The worker then becomes a builder of new comb. In addition she receives nectar and pollen brought to the hive. Pollen is stored in cells next to brood cells, while nectar is placed peripherally. Many worker bees sit over the nectar cells fanning their wings in order to evaporate the water in nectar and convert it to honey. This is the humming sound heard inside an active hive. When the evaporation is complete, the workers cap full cells with wax. At this age the worker also carries debris and dead bees out of the hive.

After two weeks of age, some young worker bees take up guard duty, staying at the entrance of the nest to inspect all incomers. Raiding bees, wasps, beetles, and flies are stung by these guards, but their stingers do not automatically pull off after stinging brittle skinned insects, so that the guards occasionally live to sting again. They also fly out to sting large animals, or people, who approach the entrance too closely. Beekeepers know that light colored polyester clothing is far less likely to antagonize bees than bright or dark colored woolens (Beekeepers also always wear shoes). Beekeepers avoid using any product with a strong odor, such as perfume, hair spray, or deodorant, when working with bees. "Smoking" a hive before entering it apparently disrupts chemical signatures, as well as possibly signaling fire and stimulating many bees to consider flight rather than attack. Bees prefer to attack the chemically strongest parts of the body, such as nostrils, mouth, and sweaty hair, hence the use of veils to cover the vulnerable head.

After three weeks of life the wax glands cease to function and the bee becomes a forager. The worker may live as long as a winter, when she is not flying about, to less than five weeks in the summer, when she literally works herself to death.

Communication

Chemical communication is the primary communication means within the hive, and each hive has its own chemical signature. Pheromones, chemical hormones which stimulate behavioral responses, identify both the individual and the colony, as well as serve to communicate courses of action. The queen bee coordinates the entire colony by emitting pheromones. The author has watched a newly mated queen bee land on the ground fully ten meters from her birth hive, away from the normal flight path, with a drone carcass nearby, stand on her head, and wave her abdomen in the air. Within seconds hundreds of worker bees had gathered. Not wishing the hive to divide by swarming at the height of the nectar season, I killed her, and ever since I have wondered how long it would have taken her to attract a support colony.

Bees also communicate by elaborate stylized movements and sounds. Some of these movements, or dances, can be seen at the hive entrance. The round dance is performed if food is less than eighty meters from the hive. The odor on the foraging worker indicates the flower in bloom, but the round dance contains no information about the direction in which to go. (Bees can see ultraviolet colors, which we cannot, but red looks dark or black to them).

The waggle dance tells other workers the direction and distance to go for food. The successful forager describes angles between the sun, hive, and food by stylized movements. Toward the sun is indicated by dancing up the hive body, away from the sun by dancing downward. Straight up means fly toward the sun, and direction left or right of vertical on the waggle run. Distance to the food source is signaled by the speed of dancing. The farther away it is, the longer the time spent in each waggle run. This waggle dance is a form of symbolic communication. These two dances, round and waggle, are illustrated in figure nine.

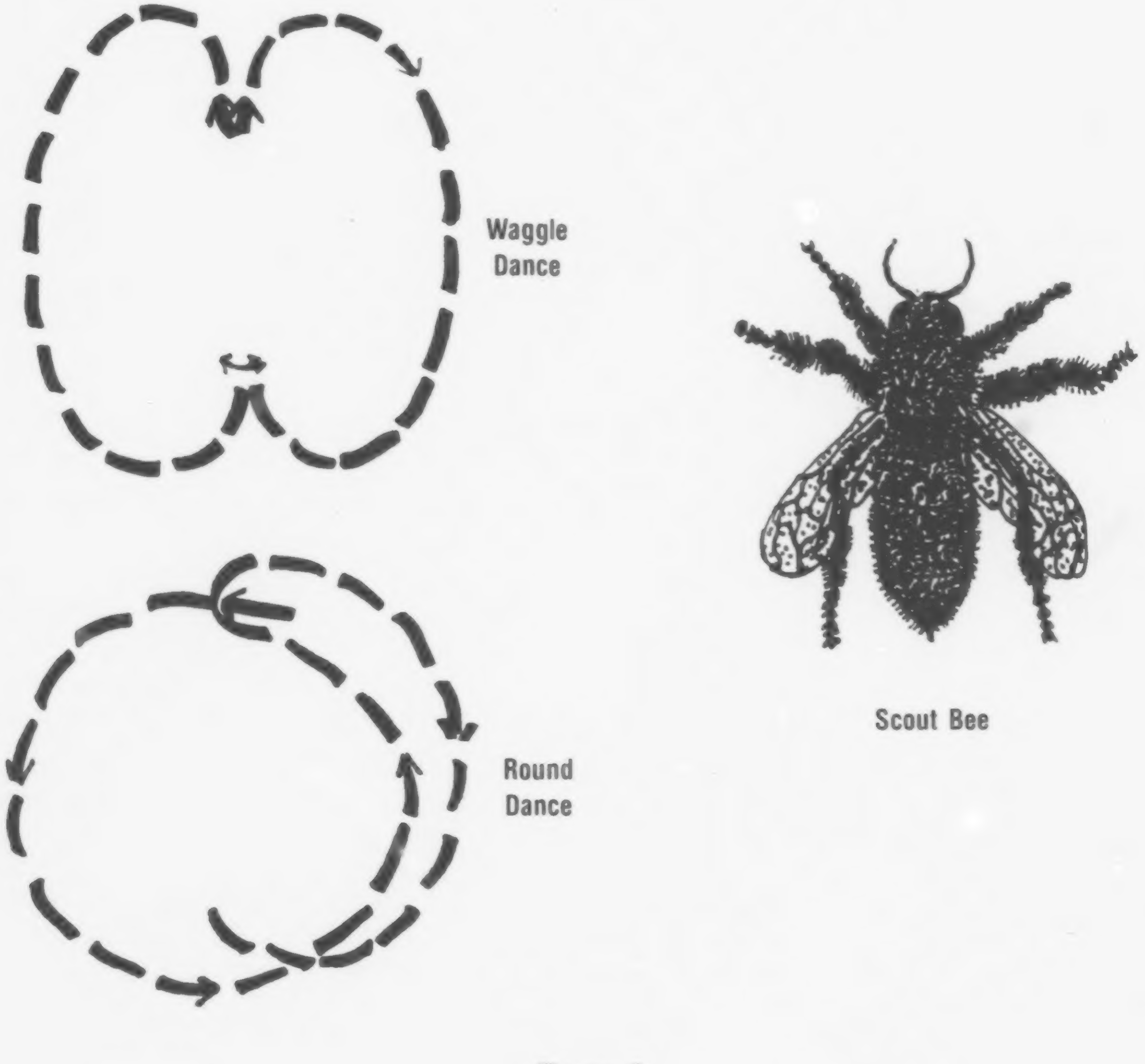


Figure 9

Steps

A. Review the following instructions on bee sting treatment. The odds are that these instructions will not have to be followed, but it is prudent to be prepared for chance stinging of an allergic individual. **Those with known generalized allergic reactions to bee or wasp stings should not participate in the observations.**

1) If someone is accidentally stung, remove them from the hive area at once. Bees smell a sting and repeatedly attack a stung area.

2) Scrape the stinger out if it is still embedded in the skin. **Do not** pull it out, for the poison sac is usually still at the end of it draining its poison into the wound. If you squeeze the poison sac, you will inject all of the remaining poison.

3) Observe the sting closely. All insect stings will cause local pain and swelling, and a bee sting can be particularly uncomfortable. This may be lessened by applying ice. If the individual does not develop a generalized allergic reaction within one hour, they most likely will not do so. If they show the following signs of a generalized allergic reaction, immediately administer an adrenalin injection and take the patient for medical treatment.

a. Hives.

b. Generalized itching.

c. Generalized redness.

d. Generalized swelling, especially around the face.

e. Difficulty breathing, either wheezing, choking, or coughing.

f. Dizziness or unconsciousness.

B. Slowly approach the entrance to the hive, staying clear of the flight path to and from the hive entrance (watch arriving and departing bees for a few minutes to determine the flight path). Use a bee veil if desired or available. **Do not step on any bees.** On a sunny, early afternoon, when large numbers of bees are absent on foraging expeditions, you should be able to approach to within one half meter of the hive entrance. Look on the ground in front of the hive, and pick up one or two drones (be certain of your identification). Remember, drones do not have stingers, so you can handle them with impunity. Return to a safe distance from the hive and study the drone.

37. Draw the drone bee in the space provided below. Label all the external structures you can identify.

38. Did hive activity change when you removed the drone? Why or why not? ______________________

____

____

C. Return to the hive, find a comfortable position to the side and away from the entrance and flight path, and study the bees on the hive's entrance platform. Note the compass direction which the hive entrance faces (beekeepers usually place them facing the south or southeast, so that early sun stimulates the workers to begin foraging). **Remember, move slowly and be patient.**

39. In the space provided below, diagram any stylized movements you observe on the entrance platform. Diagram them in relation to the hive body. Label these movements as to direction, i.e. north, south, up, down, etc. Space is provided for up to three different types of movements.

D. Follow one of the bee flight paths which seem to originate near a bee round dance. Find out where the bees are going.

40. Where are the bees going? ____

____

41. What are they gathering? Remember, not only do bees forage for nectar, but also for pollen and water (used for humidifying and cooling the hive). ____

____

42. How far from the hive is their destination? ____

Optional Activities

Students may wish to explore some other variables concerning bee behavior. They can submit their results to the instructor in a formal laboratory report.

1) Karl von Frisch won the Nobel prize for his historic work on bees in the 1930's. He proved that bees can distinguish colors (hence the assumed symbiotic relationship between the evolution of flower colors and honey bees). You can duplicate one of his experiments. Set up a small table near the bee hive. Place a piece of colored construction paper on the table. Use any color except red or black, because bees cannot see the color red. Place a petri dish containing a little sugar water on the paper. (Make sugar water by adding two parts sugar to one part water). Wait until lots of bees are coming to the petri dish. Set up another table nearby with different colored construction paper on top, and move the sugar water filled petri dish to it. Place an empty petri dish on top of the original paper and table. Where do the bees go? How can you vary this experiment? How many different sensing behaviors can you test with this experiment?

2) Mark the backs of bees clustered around a waggle or round dance with vegetable dye or paint. (One method is to drop the dye or paint while hanging over the top of the hive). Chart their movements for several hours. How many bees responded to the particular waggle dancer you observed? Do they also dance in turn?

3) Catlogue over a period of weeks the kinds of plants the bees in a particular hive visit. If other hives are nearby, mark a number of your bees to verify hive identity. What kinds of plants in your locality are pollinated by honey bees? What plants do the bees prefer? What flavor honey is produced by bees in your area (clover, locust, blackberry, tulip poplar, etc.)?

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Terminology

Students should understand the following terms and concepts prior to taking the unit review:

- agonistic
- beebread
- ethology
- imprinting
- pheromone
- royal jelly
- taxis

Review

26. Animal Behavior

Name______________________________

Date_______________________________

Match letters to numbered statements; letters may be reused.

A. Chemical communication
B. Symbolic communication
C. Imprinting behavior
D. Territoriality
E. Taxis

______ 1. A male fish attacks a plastic model of a fish with an eye and a red belly more intensely than it attacks a model with a red belly but no eye.

______ 2. A male hummingbird attacks another male hummingbird which tries to enter your back yard.

______ 3. Flies are attracted to garbage

______ 4. Baby sparrows lost their parents. You raise them, and they will not leave when adult.

______ 5. A red winged blackbird sits on a fence post warning off other blackbirds by loud song.

______ 6. A man is stung more than five times by honey bees. He flees to his house and lays panting on his bed. He looks toward the open but screened window and sees dozens of honey bees crawling on the screen.

______ 7. Mosquitos tend not to sting people covered with insect repellant.

______ 8. A red raider fish has lost a fight with another male.

______ 9. You put a duck egg under a setting chicken, and it hatches. The duck thinks it is a chicken.

______ 10. One bee found a blooming persimmon tree, and now there are thousands in the tree.

Essay

1. Describe one type of social behavior and give two examples of the communication patterns it involves.

2. Define taxis and state three types of stimuli which can cause a taxic response. ______

3. Describe conflict behavior and explain why it is thought to be important in the development of threat displays and courtship behavior. ______

Answers Found: p 355 - #3 & 7; p 356 - #4 & 9; p 361 - #1, 2, 5 & 8; p 367 - #6; p 368 - #10;
Essay #1 - p 367-368; #2: p 352-353; #3 - p 361.

27. Laboratory

Human Spacing Behavior

When you have completed this laboratory you should be able to:

1. Cite examples of human territoriality.
2. List differences in the ways males and females protect their space.
3. Consider cultural variations in spacing behavior.
4. Observe how the environment impacts spacing behavior.

Social Hierarchies

Dominance hierarchies related to reproductive, feeding, and survival competition are key features of most social groupings. Such hierarchies can be obvious, such as the pecking order in chicken flocks and the alpha male and his mate in wolf packs, or more subtle, such as can be found in flocks of parakeets and in schools of fish. Dominant individuals take their pick of limited commodities such as food, shelter, and mates. Dominance hierarchies regularly change due to youthful challenge and aging or disability of once dominant individuals. Behavioral patterns related to territoriality, such as agonistic displays and nest defense, are important in maintaining these hierarchies.

Territoriality in Vertebrates

The extent of permissible physical contact between individuals varies by species. Some animals huddle together closely, while others do not usually touch. Those species which maintain physical separation communicate with the group by sound, smell, visual signals, etc. Close contact animals include hippopotami, parakeets, and many rodents. Minimum contact animals include hawks, horses, and humans. Instinctive forms of territoriality include a balance between separation and contact typical of the species. Acceptable distance is maintained, but there also is a distance at which individuals become anxious about losing contact. Conversely, there is a critical distance beyond which strangers or other animals cannot approach without the individual wishing to fight or flee. This critical distance varies depending upon whether the animal approaching is of the same group or species.

There are individual variations in spacing behavior within a group. Dominant individuals demand more personal space, just as they command first choice of desirable commodities. Ostentatious display by wealthy humans, such as the building of very large homes, can be interpreted as a form of territorial display by dominant individuals.

Humans use knowledge of territoriality to manipulate other animals. The narrow, critical distance which separates the urge to fight or the urge to flee is used by lion trainers to manipulate the large cats. Upon entering the cage, the trainer will directly approach the lion's critical distance and keep pushing against this personal space to force the nervous cat to circle and stalk the intruder. The cat will overcome any obstacle in its path, such as jump through hoops or onto platforms, in order to stalk the trainer. The trainer intrudes into the animal's critical distance, and uses barriers such as a chair so that the animal will not directly attack, but rather circle. As soon as the lion has circled and stalked in such a way as to perform a task desired by the trainer, the lion trainer will step back from the animal's critical area, and the lion will relax. Through multiple repetitions of challenge to the lion's critical distance, the animal can be manipulated to appear trained.

Human Territoriality

Edward T. Hall of the University of Chicago studied the spacing behavior of humans, using the term proxemics to describe the ways humans use space within a group. The use of space differs among people of different cultural backgrounds and in different situations. However, Hall and his associates defined four situations standard to all cultures and peoples.

The four territories are termed intimate space, personal space, social space, and public space. The use of intimate space involves physical contact, blurred vision, smell, soft voices (speaking louder expands the distance), and awareness of body heat. Personal space, from one half to one meter, creates a small protective area, or space bubble, between oneself and others. This distance varies from hand-holding distance to arm's length, and talking is at a low to moderate level. Facial features and details are visible, but body heat is not sensed. Social distance, from one to four meters, is the distance appropriate for social gatherings and impersonal business. The entire person is readily seen, voices are moderate, and neither odor nor body heat are sensed. Public distance, from four to eight meters, is the distance for strangers or for formalities, and for voices at full capacity.

Cultural Differences

The above distances were derived from United States cultural norms. Problems in human interactions sometimes result from culturally defined differences in acceptable personal space. Since spacing responses are learned early and become automatic, individuals from different cultures can inadvertently offend. In Latin America, for instance, social distance is often no more than half a meter, similar to personal distance in the U.S. A hypothetical social conversation between a Chicagoan and an individual from Buenos Aires likely would be marked by the Chicagoan steadily backing away while the Argentine would appear to be stalking the Chicagoan around the room. Neither person is likely to understand why one is discomfited and the other feels rejected.

Middle Easterners and Americans can experience extreme communication problems because of differences in acceptable spacing behavior. Arabs and Turks recognize a much smaller personal space (although differences do exist between peoples within the region). Same sex hand-holding, bunching together, mutual kissing on the cheeks when arriving and leaving, and speaking practically mouth-to-mouth are standard behavior. Whereas Americans avoid breathing on others while speaking, Arabs require it for sincerity of communication. The American who turns his head away while speaking, or who declines the opportunity to sit close to a stranger, can quite innocently offend.

Human spacing behavior has tremendous impact, ranging from definitions of correct public behavior to the buildings we erect. American farmers, for example, scatter across the countryside in individual farmsteads, whereas Turkish peasants cluster together in villages and commute to their fields. One insists on privacy, the other needs human closeness.

The purpose of this laboratory is to determine the patterns of human spacing behavior in your school.

Pre-Lab

Supplies needed:

pencil	tape measure

Special Preparations

Students should plan to observe other students eating lunch, in a cafeteria or similar area, for three consecutive days. The observing students will require an alternate lunch period. Consequently, the only preparation required for this laboratory is scheduling.

Time Required

The human spacing behavior laboratory requires three classroom hours, in addition to preparations, discussion, and review. These classroom hours must coincide with the school lunch period.

Procedure: Hour 1

Students need all listed supplies.

During this laboratory hour the student will observe students while eating lunch and interacting during the lunch period.

Steps

A. Before lunch begins find a comfortable, unobtrusive seat, preferably with your back to the wall. Assure that one or two lunch tables are close and clearly visible. Since you can adequately observe no more than eight people interacting, one table seating six or more people will be sufficient. Measure the dimensions of the table(s), placement of chairs, and the distance to your vantage point.

1. In figure one graph provided below, sketch the overall placement of objects within the room, noting the location of your target table and vantage point.

Figure 1

2. In the figure two graph provided below, diagram on a larger scale the table(s) and chairs under observation. Indicate the scale used (1 square equals six inches, or whatever you choose).

Figure 2

3. What time does the lunch period begin? ____________

4. What is today's date? ____________

B. The lunch period has begun. Sit quietly. Notice who comes to sit at the table(s) you have chosen to observe. Notice how they seat themselves.

5. Did anyone sit down, and then almost immediately get up and move elsewhere? If so, describe or identify them.

6. Were they similar in age and sex to the others seating themselves at the table(s)? ____________

7. In figure two, diagram positions of those at the table(s) after five minutes have elapsed.

8. In the space provided below, provide a description of each person under observation. Include name, if known, age (or estimated age), sex, size, ethnic background, dress, etc.

9. In the space provided below, describe the lunch of each person under observation.

10. Observe them while eating. Do they eat only from their individual lunches or do they trade and share food?

11. If there is any food sharing, in the space provided below, describe exactly what is shared and how. Also include the why of the sharing if it is obvious.

12. Note how they place their feet. Ten minutes after they have seated themselves at your observation table, diagram the position of their feet in relation to each other, and the table, in the figure three graph provided below.

Figure 3

13. Note the position of their hands and arms. Fifteen minutes after they have seated themselves at your observation table, diagram the position of their hands and arms in relation to each other, and the table, in the figure four graph provided below.

Figure 4

14. In the space provided below, identify who talks with whom at the table(s). Note everything you can of their interactions during the time they are at the table(s), such as moving chairs close or away, leaning toward or away from each other, etc.

15. In the space provided below, identify the time and the order at which people leave the table(s). Note if they leave separately or together. Note if they leave the lunch room with someone from the same table, or with someone from a different table.

Procedure: Hour 2

Students need all listed supplies.

During this laboratory hour you will again observe a group of students eating lunch and interacting.

Steps

A. Repeat the procedures of hour one of this laboratory, excepting question one/figure one (placement of the table under observation relative to objects within the room at large). If furniture has been moved about since your first hour of observations, note those changes on figure one. Observe the same table(s) from the same observation seat as before. Enter your data in the format provided below.

16. In the figure five graph provided below, diagram the table(s) and chairs under observation. Indicate the scale used (1 square equals six inches, or whatever you choose).

Figure 5

17. What time does the lunch period begin? ____________

18. What is today's date? ____________

B. The lunch period has begun. Sit quietly (valid results require that the observer be unobtrusive). Notice who comes to sit at the table(s) you have chosen to observe. Notice how they seat themselves.

19. Did anyone sit down, and then almost immediately get up and move elsewhere? If so, describe or identify them.

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20. Were they similar in age and sex to the others seating themselves at the table(s)? ____________

21. In figure five above, diagram the positions of those at the table(s) after five minutes has elapsed.

22. In the space provided below, provide a description of each person under observation. Include name, if known, age (or estimated age), sex, size, ethnic background, dress, etc.

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23. In the space provided below, describe the lunch of each person under observation.

24. Observe them eating. Do they eat only from their individual lunches or do they trade and share food?

25. If there is any food sharing, in the space provided below, describe exactly what is shared and how. Also include the why of the sharing if it is obvious.

26. Note how they place their feet. Ten minutes after they have seated themselves at your observation table, diagram the position of their feet in relation to each other, and the table, in the figure six graph provided below.

Figure 6

27. Note the position of their hands and arms. Fifteen minutes after they have seated themselves at your observation table, diagram the position of their hands and arms in relation to each other, and the table, in the figure seven graph provided below.

Figure 7

28. In the space provided below, identify who talks with whom at the table(s). Note everything you can of their spacing interactions during the time they are at the table(s), such as moving chairs close or away, leaning toward or away from each other, etc.

29. In the space provided below, identify the time and the order at which people leave the table(s). Note if they leave separately or together. Note if they leave the lunch room with someone from the same table, or with someone from a different table.

Procedure: Hour 3

Students need all listed supplies.

During this laboratory hour you will again observe a group of students eating lunch and interacting.

Steps

A. Repeat the procedures of hours one and two of this laboratory. Observe the same table(s) from the same observation seat as before. Enter your data in the format provided below.

30. In the figure eight graph provided below, diagram the table(s) and chairs under observation. Indicate the scale used (1 square equals six inches, or whatever you choose). As during hour two, note placement of the observation table relative to the entire room only if furniture has been rearranged.

Figure 8

31. What time does the lunch period begin? ____________

32. What is today's date? ____________

B. The lunch period has begun. Sit quietly (for valid results you must be unobtrusive). Notice who comes to sit at the table(s) you have chosen to observe. Notice how they seat themselves.

33. Did anyone sit down, and then almost immediately get up and move elsewhere? If so, describe or identify them.

34. Were they similar in age and sex to the others seating themselves at the table(s)? ____________

35. In figure eight diagram positions of those at the table(s) after five minutes has elapsed.

36. In the space provided below, provide a description of each person under observation. Include name, if known, age (or estimated age), sex, size, ethnic background, dress, etc.

37. In the space provided below, describe the lunch of each person under observation.

38. Observe them eating. Do they eat only from their individual lunches or do they trade and share food?

39. If there is any food sharing, in the space provided below, describe exactly what is shared and how. Also include the why of the sharing if it is obvious.

40. Note how they place their feet. Ten minutes after they have seated themselves at your observation table, diagram the position of their feet in relation to each other, and the table, in the figure nine graph provided below.

Figure 9

41. Note the position of their hands and arms. Fifteen minutes after they have seated themselves at your observation table, diagram the position of their hands and arms in relation to each other, and the table, in the figure ten graph provided below.

Figure 10

42. In the space provided below, identify who talks with whom at the table(s). Note everything you can of their spacing interactions during the time they are at the table(s), such as moving chairs close or away, leaning toward or away from each other, etc.

43. In the space provided below, identify the time and the order at which people leave the table(s). Note if they leave separately or together. Note if they leave with someone else from the same table, or with someone from a different table.

44. In the space provided below, describe any changes which took place in group composition at your observation table(s) during the three days. What might account for any change, or lack of change?

45. Study your three days of diagrams of body placements at the table(s). What is the average space maintained between individuals?____________

46. Were there great exceptions to this average spacing? If so, what were they?

47. Compare your findings with others in the class. Which sex, males or females, maintained a greater average spacing between individuals? What was the difference? Why this gender difference?

48. Compare your findings with others in your class. Which age group, older or younger, maintained a greater average spacing between individuals? What might account for this difference?

49. If your high school is composed of individuals with different ethnic backgrounds, what differences in space behavior, other than sex or age differences, did you note?

50. If your table(s) had mixed seating, that is both males and females together, what deviations from gender averages did you observe?

51. Aside from differences resulting from age, did body size differences affect seating and spacing behavior?

Describe

52. Review your observations. In the space provided below, what conclusions can you draw regarding spacing behavior in your school?

53. Reflecting upon spacing behavior differences relative to age, size, sex, and ethnicity, can you think of other human behavior patterns which might be objectively measured and quantified?

Optional Activities

Students may wish to explore some variables concerning human spacing behavior. They can submit their results to the instructor in a formal laboratory report.

1). Choose any other public place in which a large number of people is likely to congregate, such as a library, a bus or train depot, or a park. Repeat this experiment. Do people preserve personal space in a manner similar to the students you observed during their lunch period?

2). Repeat this laboratory experiment in a classroom. In what order do students occupy seats in a class when free choice of seating is available? Do you note patterns possibly related to age, size, sex, and ethnicity? Does seating affect whether or not a student participates in class discussions?

3). Observe people in a public place. Note the behavior of those who wish to retain their position or seat, yet need to leave the area for a few minutes. What types of markers or behaviors do they use to preserve their territory? What types of markers or behaviors are most effective in preserving personal territory? Make a graph of markers ranging from the very personal, such as a piece of clothing, to the impersonal, such as library books, and correlate the degree of personalness with the length of time the marker was effective. What conclusions can you reach?

4). Make observations on personal spacing at a large-crowd event, such as a parade or concert. Compare these data with your observations on smaller groups, such as the lunch period in this laboratory. Suggest hypotheses for any differences you notice.

Resources

Hall, Edward T. *The Hidden Dimension*. Doubleday: Garden City, New York, 1966.

Motley, Michael T. "Slips of the Tongue." *Scientific American*, September 1985.

Ramachandran, Vilayanur S. and Stuart M. Anstis. "The Perception of Apparent Motion." *Scientific American*, June 1986.

Sommer, Robert. *Personal Space*. Prentice-Hall: Englewood Cliffs, New Jersey, 1969.

Wilson, E. O. *Sociobiology: The New Synthesis*. Harvard University Press: Cambridge, Massachusetts, 1975.

Terminology

Students should understand the following terms and concepts prior to taking the unit review.

critical distance

proxemics

Review

27. Human Spacing Behavior

Name____________________________

Date_____________________________

Essay

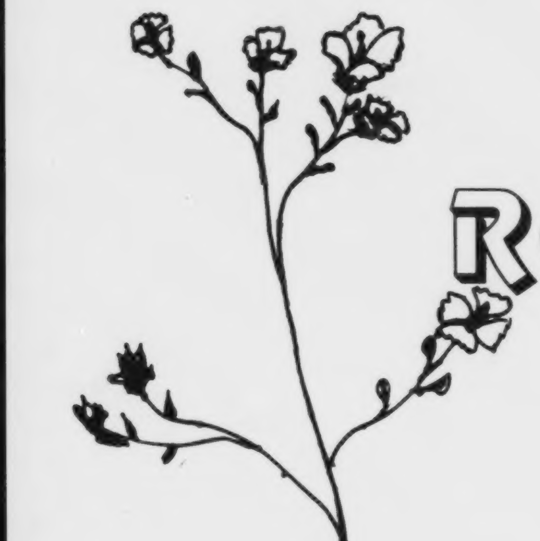
1. Discuss territoriality and the behaviors it engenders.

2. Discuss proxemics in humans.

3. Consider the layout of private homes, businesses, public buildings, outdoor recreational facilities, and other users of space in the area where you live. To what extent and how is this layout influenced by culturally defined human spacing behavior?

Answers Found: thought

Plant Growth Relationships



28. Laboratory

Plant Growth

When you have completed this laboratory you should be able to:

1. Explain how the basic structure and growth pattern of vascular plants are adapted to their functions.
2. Determine the locus of growth of roots, stems, and leaves.
3. Demonstrate the role of auxins in phototropism and apical dominance, and list at least two other roles of auxins.
4. Determine the factors that control cell elongation and seed germination.
5. Demonstrate what is meant by the terms short-day, long-day, and day-neutral plants.
6. Give evidence that photo-periodic flowering responses are controlled by changes in phytochromes.

Purpose

The purpose of this laboratory is to introduce the student to the study of plant growth. Live plant laboratories such as this require time for plants to respond to conditions placed upon them, and timing is an important aspect of such studies. This laboratory actually is a cluster of seven mini-experiments begun and concluded over a timed and separated set of laboratory sessions.

Locus of Plant Growth

Plant growth is influenced by many factors. Before observing how environmental changes influence plant growth, it is essential to know what parts of a plant grow, and how fast they grow in relation to other parts. The first experiment, begun in hour one of this laboratory and concluded in hour five, pinpoints the locus of plant growth in stems, leaves, and roots.

Phototropism

The second experiment in this laboratory, begun in hour one and concluded in hour six, repeats Charles Darwin's classic experiment on phototropism. Darwin, attempting to identify the most physiologically active site in a plant, studied the effects of gravity and unidirectional light on plant movement. He concluded, in *The Power of Movements in Plants*, that "when seedlings are freely exposed to a lateral light some influence is transmitted from the upper to the lower part causing the latter to bend." Today we know that this "influence" is exerted by plant hormones called auxins. They stimulate cell elongation and RNA transcription within the cell which are then followed by an increase in the biosynthesis of proteins.

Light, Gibberellin, and Growth

The third experiment, begun in hour one, continued in hours seven and eight, and concluded in hour nine, studies how light rays of specific frequency affect plant growth. It also demonstrates how gibberellin, a plant-growth regulator, influences plant growth. More than fifty gibberellins have been identified, and characteristically they stimulate cell elongation, tissue differentiation, and bolting.

Control of Cell Elongation

The fourth experiment, begun in hour two and concluded in hour three, studies some chemical regulators involved in plant cell elongation. Coleoptiles, leaf growth tips, will be excised and placed in various test tubes. Separating these parts from the rest of the plant avoids endogenous auxins or other regulatory chemicals. An auxin, IAA (indole acetic acid), will be applied in varying amounts to different test tubes, along with other synthetic auxins and auxin inhibitors. It is not yet fully known how auxins regulate cell elongation, but factors such as increased cell permeability to water, reduction in cell wall pressure, and an increase in wall synthesis have been observed. Students will graph some factors influencing growth versus IAA concentration.

Seed Germination

Seed germination varies considerably in response to light. Some seeds require light to germinate, light inhibits germination in other seeds, and, in still others, germination is associated with a photo-periodic response. Temperature also may interact with light to influence the germination of some seeds.

In the fifth experiment, begun in hour four and concluded in hour six, lettuce seeds will be germinated in response to specific wavelengths of light. Because imbibition of water modifies the light germinating response of lettuce seeds, this factor was standardized before the start of the experiment.

Additionally, three plant hormones and their effects on lettuce seed germination are tested. An auxin, IAA, which stimulates plant cell elongation and growth, a plant growth hormone, GA (gibberellin), which also stimulates cell elongation, and a cytokinen, kinetin, which stimulates cell division, are all used to test lettuce seed germination.

Control of Bud Dormancy

In the sixth experiment, begun in hour one, continued in hour eight, and concluded in hour nine, the student will consider the effects of two plant hormones on bud development. This is an elegantly simple experiment in which the action of a plant auxin, IAA, and a plant growth regulator, GA, are considered both separately and jointly.

Flowering in *Pharbitus nil*

In the seventh experiment, begun in hour seven and concluded in hour ten, the effect of day/night length on the stimulation of flowering in morning glories, *Pharbitus nil*, is studied by varying the amount of light they receive. Different plants flower variously in response to the amount of light they receive. They may be short-day or long-day plants. Some plants flower in response to the amount of dark they receive, such as morning glories. They may be short-night or long-night plants. Some plants are not affected by the amounts of light or dark they receive. They are called day-neutral plants.

In the second part of this seventh experiment, the role of florigen, a plant flowering regulator, is studied indirectly. It is believed that florigen is produced in plant leaves and travels to the nodes to stimulate flowering. It was described and named by M. Kh. Chailakhyan, a Russian scientist during the 1930's. Since it has not yet been successfully isolated, some scientists question the existence of such a chemical. However, it apparently is influenced by several other plant hormones, such as IAA and by phytochromes, pigments which respond to light intensity and periodicity, and especially by the level of GA. Since GA production is influenced by day/night length, florigen production also is influenced by day/night length. In addition, florigen production seems to be stimulated variously by different light wavelengths. The issue of florigen is an excellent area for further research.

Pre-Lab

Supplies needed:

Access To

greenhouse
vial cabinet
controlled environment chamber
3 cabinets, closets, or small darkrooms, equipped with plant stands

Equipment

600 ml. beaker
8 x 20 cm. glass plate
2 rubber bands
12 test tubes
test tube rack
9 petri dishes
ruler
razor
40 shell vials
vial tray
10 plant pots
flashlight
marking pen
5 μl pipet
plant grid marker
sponge

Materials

fingerprint ink
paper toweling
filter paper
distilled water
vermiculite
0.1% IAA in lanolin
50% ethanol with 50 μg/ml GA
50% ethanol
GA, 1 ppm in ethanol
1% GA in lanolin
0.1 ppm kinetin
green cellophane
piece of paper
0.025 M. maleate
10% sucrose water solution
IAA, 1 μg/ml ethanol solution
GA, 50 μg/ml ethanol solution
0.1 M. calcium chloride
1 M. mannitol water solution
cycloheximide, 100 μg/ml solution
10^{-3} M. potassium cyanide
1 ppm IAA in ethanol
1% IAA in lanolin
lanolin
aluminum foil

Living Materials

1 pot with 4-6 bean seedlings
40 soaked corn seeds
60 corn seedlings
48 Alaska pea seeds
24 soaked Alaska pea seeds
24 soaked dwarf pea seeds
3 germinating pea seedlings
450 soaked (16 hours in the dark) lettuce seeds
10 day old potted *Pharbitus nil* (morning glory) seedlings

Special Preparations

1) Because of the deferred observation times in this laboratory, care must be taken when scheduling the first hour. Allow seven days between the seventh and eighth hours of this laboratory, and five days between the eighth and ninth hours of this laboratory. The tenth hour cannot be concluded until at least twenty-eight days after the beginning of this laboratory, or three weeks after the seventh hour, whichever is easier to schedule. In other words, the entire laboratory will be spaced over a one month period, but the initial seven hours may be completed in seven consecutive laboratory sessions.

2) Students must have access to a greenhouse to conduct this laboratory. Also, it is difficult for the instructor to have necessary living plants ready for use, if a greenhouse is not available. There are many models of inexpensive glass or plastic sheeting greenhouses available for those schools lacking a standard greenhouse.

3) Controlled environment chambers are available from many biological supply houses. The controlled environment chamber should have a timer for light control. If one did not come with the school's environment chamber, they are relatively easy to purchase and install separately. For this laboratory, the controlled environment chamber must be placed in a room or closet in which the only light received by the plants will be from the chamber itself.

4) Vial cabinets are available from many biological supply houses. Obtain a vial cabinet of sufficient size for the entire class. Each student group will need to place one tray with 40 vials in the cabinet. Of currently available vial trays, the closest in size holds up to 45 vials.

5) Of the three cabinets, closets, or small darkrooms, one should have no lighting, one should have dim red lighting, and the third should have fluorescent lighting. The entrance to each should be hung with blackout cloth to keep out extraneous light when people enter or leave. If space is a problem, the three enclosures may consist of light protected shelving in the classroom proper. However, this experiment is much more reliable if students are able to enter the areas and remain inside to manipulate the plants. Blackout cloth is essential because very small amounts of extraneous light may be enough to invalidate results. Dim red lighting may be installed either by using red bug lights, or by using grow lights over which red cellophane has been taped at sufficient distance so as not to melt. Plant stands within the enclosures may either be built-in or borrowed mobile plant stands from classrooms.

6) Plant grid markers in the form of rubber stamps are available from many biological supply houses. Plant materials such as leaves may be backed by a damp sponge while being stamped with a plant grid marker coated with fingerprint ink. Fingerprint ink is readily available.

7) It is convenient to purchase plant auxins as a kit. Plant regulator kits containing seventeen auxins in 100 mg. amounts are available from chemical supply houses. Those auxins not used in this laboratory will be used in either laboratory twenty-nine or thirty. Or, you may prefer to purchase each auxin or regulator separately.

8) Cycloheximide, if purchased separately, is sold specifically as 3-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl) glutarimide. Kinetin, if purchased separately, is sold as 6-furfurylaminopurine. Maleate also is known as maleic acid hydrazide and, if purchased separately, is sold as 1,2-dihydro-3,6-pyridazinedione.

9) Have the chemicals required for hour two available in stock erlenmeyer flasks, with a 5 ml. pipet in each flask.

10) To add auxins to lanolin, heat the lanolin only enough to melt it, and stir in the auxin. 0.1% IAA (indole 3-acetic acid) in lanolin may be prepared by dissolving 100 mg. IAA in 2 ml. absolute ethyl alcohol and stirring this solution into 100 g. lanolin paste. 1.0% IAA in lanolin may be prepared in the same fashion, in this case dissolving 1 g. IAA. GA (gibberellic acid, also called gibberellin from the fungus from which it was first isolated) in lanolin is prepared in a similar manner. This makes enough IAA and GA in lanolin for classes for several years. Also, growth regulator paste kits, which contain all the above auxins in lanolin, are available in many biological supply catalogues and can be purchased "ready made."

11) Those seeds which need to be soaked before using, except for the lettuce seeds, are to be placed in water twelve to twenty-four hours prior to the laboratory session. Plant bean seeds in pots three weeks before the laboratory session. Plant corn seedlings two weeks before the laboratory session, and start pea seedlings ten days prior to the laboratory session.

12) The morning glories are used in a group laboratory on stimulation of flowering. Students will monitor the experiment, but the experiment itself will be done by the class as a whole. The instructor will need to start 13 pots of morning glories for each class ten days before the laboratory session under long-day conditions in an environment chamber (16 hours light and 8 hours dark, repeating). After eight days, and two days before the hour seven procedure, change the lighting conditions to continuous, 24-hour light.

13) **Fourteen students will be scheduled for brief laboratory work during off-hours in connection with the morning glory flowering experiment. All students will be required to return to the laboratory two to four hours after hour six in order to observe the results of the phototropism experiment.**

14) Any type of garden pot may be used in these experiments. Clay or plastic pots are convenient for larger plants and those plants which will grow in a pot for more than three weeks. Peat pots are convenient for starting plants and sprouting seeds.

Time Required:

The plant growth laboratory requires ten classroom hours, in addition to preparations, discussion, and review. The laboratory sessions take place over a period of one month, even though most of the laboratory sessions are scheduled during the first seven classroom hours. During the month-long period plants must be watered and tended.

Procedure: Hour 1

Students need the following: pot with bean seedlings, plant grid marker, fingerprint ink, sponge, 600 ml. beaker, glass plate, paper toweling, distilled water, 3 germinating pea seedlings, rubber band, aluminum foil, 40 shell vials, vial tray, vermiculite, 40 soaked corn seeds, 10 pots, 8 soaked Alaska pea seeds, 8 soaked dwarf pea seeds, and 48 Alaska pea seeds.

Additionally, students need access to all structures listed under "Access To" in the pre-laboratory instructions.

During this laboratory hour students will set up experiments on stem and leaf growth, root growth, phototropism, light and gibberellin, and control of bud dormancy.

Steps

A. **Stem and leaf growth:** an experiment on the locus of plant growth. Obtain a pot which contains four to six bean seedlings grown in the light. Label the pot with your name and today's date. Examine the seedlings carefully and note the number of visible leaves and nodes. Coat a plant grid marker with fingerprint ink, and moisten a sponge. Select two of the seedlings and mark them with the grid marker from the ground to the apex. Use the moistened sponge to support the seedlings during this operation. Depending on the plant grid marker available to you, your lines will be either 1 or 2 mm. apart. Select two other seedlings and apply the grid marker to the smallest visible leaf (as long as it is greater than 5 mm. long) on each plant. Return the plants to the greenhouse for five days.

The above experiment may also be done using India ink and a fine pen with ruler. In that case allow an entire hour for the step A procedure.

B. **Root growth:** an experiment on the locus of plant growth. Prepare a moist chamber by lining the sides of a 600 ml. beaker with thoroughly moistened paper toweling. Completely cover an 8 x 10 cm. glass plate with filter paper soaked with distilled water. Label the beaker with your name and today's date.

Select a healthy, straight, germinating pea seedling and wipe off excess moisture. Mark the root, starting at the tip and continuing for one centimeter, with the plant grid marker and fingerprint ink, again using a moist sponge for support. Do this for three roots. Mark three more roots with a single mark one centimeter from the tip.

Attach the seedlings to the glass plate by slipping them through a rubber band attached to the glass plate. Cover the beaker with aluminum foil and place it in your locker or drawer for five days.

C. **Phototropism:** a classic experiment first performed by Charles Darwin. Fill 40 shell vials with wet vermiculite, and plant in each a single, soaked corn seed, radicle down. Place the vials in the vial tray, and label the tray with your name and today's date. Store this in the vial cabinet for six days.

D. **Light, Gibberellin, and Growth:** an experiment testing the result of adding the growth regulator gibberellin to plants. (Phosfon, a chemical with an impact opposite to that of gibberellin, is used by the horticultural trade to make plant growth more compact on ornamental plants.)

Fill six pots with wet vermiculite. To three pots add 8 soaked Alaska pea seeds, and to the other three pots add 8 soaked dwarf pea seeds. Label each pot with your name and date, and seed type. Place one pair in the enclosure with fluorescent light, one pair in dim red light, and the third pair in the dark enclosure. Make sure the pots stay moist, but otherwise leave them for eight days.

E. **Control of Bud Dormancy:** an experiment to determine bud dormancy control. Fill four pots with good potting soil or vermiculite and plant them with 12 Alaska pea seeds each. Water them and make sure they stay moist, but otherwise leave them in the greenhouse for fifteen days.

Procedure: Hour 2

Students need the following: 0.025 M. maleate, 10% sucrose water solution, IAA, 1 μg/ml ethanol solution, GA, 50 μg/ml ethanol solution; 0.1 M. calcium chloride solution, 1 M. mannitol water solution, cycloheximide, 100 μg/ml solution, 10^{-3} M. potassium cyanide solution, distilled water, 12 test tubes, test tube rack, razor, and 60 corn seedlings.

During this laboratory session you will set up an experiment to determine the control of cell elongation in plants. Corn coleoptile sections will be used. This procedure has been selected because growth is due entirely to cell elongation stimulated by growth hormones, and such hormones will be added to the separated coleoptiles.

Steps

A. Number the test tubes one through twelve, and place them in a test tube rack which you have labeled with your name and today's date. Use the figure one chart as a guide, and fill the test tubes with the same solutions listed in the chart. When filled, all test tubes should contain exactly 5 ml. of liquid.

Test Tube Number

Solution Add in ml.	1	2	3	4	5	6	7	8	9	10	11	12
Maleate, 0.025M	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sucrose, 10%	1	1	1	1	1	1	1	1	1	1	1	1
IAA, 1 μg/ml	0	0.5	0.5	0.5	0.5	2.5	0.5	0	0.5	0.5	0.5	0.5
GA, 50 μg/ml	0	0	0	0	0	0	0	0.5	0	0	0	0
CaCl, 0.1M	0	0	0	0	0	0	0	0	1.5	0	0	0
Mannitol, 1M	0	0	0	0	0	0	0	0	0	1	0	0
Cycloheximide, 100 μg/ml	0	0	0	0	0	0	0	0	0	0	0.25	0
KCN, 10^{-3}M	0	0	0	0	0	0	0	0	0	0	0	1.5
H_2O	3.5	3	3	3	3	1	4	3	1.5	2	2.75	1.5

Figure 1

B. Select 60 corn seedling coleoptiles between 2.5 and 3.3 cm. in length and carefully cut a 10 mm. section from each. Place five sections in each test tube. Place the test tubes and rack in the greenhouse for twenty-four hours.

Procedure: Hour 3

Students need the following: test tubes filled with corn coleoptiles from hour two laboratory, and a ruler.

During this laboratory hour you will measure coleoptile sections and determine the effects of different substances on decapitated coleoptile growth.

Steps

A. Carefully measure the corn coleoptile sections with a ruler.

1. Enter your finds in the figure two chart below.

Test Tube Number

Coleoptile Length in mm.	1	2	3	4	5	6	7	8	9	10	11	12
#1												
#2												
#3												
#4												
#5												

Figure 2

2. Construct a growth versus IAA concentration graph in the figure three graph on page 410.
3. In the space provided below, explain the effects of chemicals other than IAA on growth.

Figure 3

4. Why is it important that the coleoptiles be severed from the plant in order to accurately measure chemical stimulation of cell elongation?

5. What can you infer from these results concerning the requirements for cell elongation?

Procedure: Hour 4

Students need the following: 9 petri dishes, filter paper, 3 ml. distilled water, 3 ml. 1 ppm GA, 3 ml. 1 ppm IAA, 3 ml. 0.1 ppm kinetin, 450 lettuce seeds soaked for 16 hours in the dark, flashlight, green cellophane paper, rubber band, and aluminum foil.

You also will use two cabinets, closets, or small darkrooms which are dark and equipped with red light.

During this laboratory hour you will set up an experiment to study the effects of light on lettuce seed germination. You also will determine the effects of three plant hormones, an auxin (IAA), a growth regulator (GA), and a cytokinen (kinetin) on seed germination.

Steps

A. Place filter paper in the bottom of 9 petri dishes. Label dishes #1 through #9, and put your name and today's date on each of them. To the dishes add:

dishes #1 - 5, add 3 ml. distilled water;
dish #6, add 3 ml 1 ppm GA;
dish #7, add 3 ml. 1 ppm IAA; and,
dishes #8 & 9, add 0.1 ppm kinetin.

B. Attach green cellophane to a flashlight with a rubber band, so that light from the flashlight shines through the green cellophane. You will use the shielded flashlight to work in the dark. The following steps must otherwise be conducted in the dark. When moving petri dishes and lettuce seeds from one light source to another, as detailed below, cover the entire petri dish with aluminum foil to protect the dish from extraneous light which might invalidate your experimental results.

1). Working with the green cellophane filtered flashlight, place in each of the nine dishes 50 lettuce seeds that have been soaked for 16 hours in the dark.

2). Place dishes #1, 6, 7 & 8 immediately in the dark.

3). Expose dishes #2, 3, 4, 5 & 9 to three minutes of red light. Place #2 & 9 in the dark.

4). Expose dishes #3, 4 & 5 to seven minutes of white light or bright sunlight. Place #3 in the dark.

5). Expose dishes #4 & 5 to three more minutes of red light. Place #4 in the dark.

6). Expose dish #5 to 6 minutes of white light or bright sunlight. Place #5 in the dark.

7). Leave the nine petri dishes in total darkness for 48 hours.

Procedure: Hour 5

Students need the following: laboratory set ups from hour one, steps A and B (stem, leaf, and root growth) and a ruler.

During this laboratory session you will determine the locus of plant growth in stems, leaves, and roots.

Steps

A. Retrieve the light grown seedling pot from the greenhouse. Measure the distance between each mark on the stems.

6. How many millimeters apart were the original marks? ____________

7. Write in the chart, figure four, the distance between each mark on the stems of the seedlings. Average results, and enter the average in the chart.

Distance	Seedling #1	Seedling #2	Seedling #3	Average
1. Distance from ground to first mark				
2. Distance from first to second mark				
3. Etc.				
4.				
5.				
6.				
7.				
8.				

(Continued)

Distance	Seedling #1	Seedling #2	Seedling #3	Average
9.				
10.				
11.				
12.				
13.				
14.				
15.				
16.				
17.				
18.				
19.				
20.				
21.				
22.				
23.				
24.				
25.				
26.				
27.				
28.				
29.				
30.				
31.				
32.				
33.				
34.				
35.				

Figure 4

8. Chart growth pattern of the average seedling stem, marking to scale final placement of the grid marks. Use the following figure five graph.

Figure 5

9. Where did stem growth occur? ______________________________

10. What parts of the stem achieved the maximum growth rate? ______________________________

B. Measure the distance between each mark on the leaves of the three seedlings.

11. Record in the chart, figure six, the distance between each mark on the leaves of the seedlings. Average results, and enter the average in the chart.

Distance	Seedling #1	Seedling #2	Seedling #3	Average
1. Distance from stem to first mark				
2. Distance from first to second mark				
3. Etc.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13.				
14.				
15.				
16.				
17.				
18.				
19.				
20.				

Figure 6

12. Diagram the growth of the average seedling leaf in the graph, figure seven.

Figure 7

13. Where did leaf growth occur? ______________________________

____

14. What parts of the leaf achieved the maximum growth rate? ______________________________

____

____

C. Measure the distance between the marks on the pea seedling roots.

15. Record in the chart, figure eight, the distance between each mark on the seedling roots. Average results, and enter the average in the chart.

Distance	Seedling #1	Seedling #2	Seedling #3	Average
1. Distance from root tip to first mark				
2. Distance from first to second mark				
3. Etc.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13.				
14.				

(Continued)

Distance	Seedling #1	Seedling #2	Seedling #3	Average
15.				
16.				
17.				
18.				
19.				
20.				
21.				
22.				
23.				
24.				
25.				
26.				
27.				
28.				
29.				
30.				
31.				
32.				
33.				
34.				
35.				

Figure 8

16. Diagram growth of the average seedling stem in the graph, figure nine.

Figure 9

17. Where did root growth occur? ____

____

18. What parts of the root achieved the maximum growth rate? ____

____

____

Procedure: Hour 6

Students need the following: set ups from hour one step C (phototropism) and hour four step B (lettuce seeds in petri dishes), razor, 0.1% IAA in lanolin, and aluminum foil.

During this laboratory hour you will continue Darwin's experiment on phototropism by exposing corn seedlings to unidirectional white light. You also will record the germination rate of lettuce seeds under varying light and chemical conditions. **Arrangements must be made for students to return to the laboratory two to four hours after the regularly scheduled hour in order to complete step C.**

Steps

A. Retrieve from the vial cabinet the tray of shell vials filled with corn seedlings. Select the twenty-five healthiest appearing seedlings and discard the remainder. Arrange these in the tray in five groups of five each. Mark the groups as #1 through #5. Handle the groups as follows:

- #1. Leave intact.
- #2. Decapitate (cut off growth tip).
- #3. Decapitate and add 0.1% IAA in lanolin to stumps.
- #4. Cover growth tips with tiny foil caps.
- #5. Cover stem bases with tiny foil cylinders.

B. Expose the tray for twenty minutes to unidirectional white light at right angles to normal growth direction. Place the tray in red light for two to four hours.

C. **Students must return in two to four hours in order to determine the extent, direction, and location of bending.** This step, being qualitative, should take only a few minutes.

19. In the chart, figure ten, note phototropic reactions.

Reaction	Group #1	Group #2	Group #3	Group #4	Group #5
Direction State whether bending toward or away from the light, or none.					
Extent Mark the groups from "A" to "E" in terms of most to least bending.					
Location Where does the plant bend, stem near the root, in the middle, etc.?					

Figure 10

20. Explain your results

D. **This step will be completed after step B, during the normal laboratory hour.** Obtain the nine petri dishes with lettuce seeds set up in hour four of this laboratory. Observe the dishes.

21. Record in the chart, figure eleven, the number of germinated seeds out of fifty placed in each dish, and determine the percent germination.

Dish	Number Germinated/50	Percent Germination
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		

Figure 11

22. What combination of light led to the highest percent germination of lettuce seeds? Why?

23. What effects did the addition of IAA have on lettuce seed germination?

24. What effects did the addition of GA have on germination?

24. What effects did the addition of the cytokinen, kinetin, have on seed germination?

Procedure: Hour 7

Students need the following: the set up from laboratory hour one step D (light, gibberellin and growth, flashlight with green cellophane attached by rubber band, ruler, piece of paper, μl pipet, 50% ethanol containing 50 μl/ml. GA, and 50% ethanol.

Students need access to all three controlled light enclosures, namely: dark, red light, and fluorescent light.

The whole-class experiment on flowering requires 13 pots with three each ten-day old morning glory *(Pharbitus nil)* seedlings (see item 12 under special preparations).

During this laboratory hour you will continue the experiment on light, gibberellin and growth begun in hour one of this laboratory. You also will participate in the set up a whole-class experiment on the effects of light on flowering in *Pharbitus nil.*

Steps

A. **Light, gibberellin, and growth:** examine the six pea seedling pots started during hour one of this laboratory and placed in the dark, fluorescent light, and red light enclosures. When examining the plants in the darkroom, use a flashlight covered with green cellophane to produce a dim green light. For each plant, note the length of the stem, the number of nodes, the number of leaves, the size of the largest leaf, and the shape of the leaf apex.

26. Record your observations in the figure twelve chart below.

Growth	Dark		Red		Fluorescent	
	Alaska	Dwarf	Alaska	Dwarf	Alaska	Dwarf
How many of 8 germinated?						
Average Stem Length						
Average Node Number						
Average Leaf Number						
Largest Leaf Size						
Shape Leaf Apex						

Figure 12

27. What effects did the fluorescent light, dim red light, and dark have on plant growth? ____________

B. To half the plants in each pot add a 10 μl drop pf 50% ethanol containing 50 μg/ml GA. Apply the 10 μl drop directly to the base of each plant. To the others add 10 μg of 50% ethanol. Mark the two groups in each pot by dividing them with a labeled paper strip. Return the pots to their original enclosures for seven additional days.

C. **Flowering in *Pharbitus nil:*** an experiment to determine the critical night length of morning glories, and also to determine the rate of florigen movement from the leaf. The class as a whole will coordinate efforts to demonstrate these phenomena.

1). **Critical night length:** obtain seven pots with three 10-day old *Pharbitus nil* seedlings. These seedlings have been raised in the environment chamber under long-day conditions (16 hours light, 8 hours dark, repeating), until two days before class, and then under continuous light. Label the pots "critical night length," numbering them one through seven, and place them in the darkroom. Schedule **seven** students to return the pots to long-day conditions in the environment chamber after the following hours of dark.

Person Responsible

Pot #1 — after 8 hours dark. ______________________________

Pot #2 — after 9 hours dark. ______________________________

Pot #3 — after 10 hours dark. ______________________________

Pot #4 — after 11 hours dark. ______________________________

Pot #5 — after 12 hours dark. ______________________________

Pot #6 — after 14 hours dark. ______________________________

Pot #7 — after 16 hours dark. ______________________________

Leave the pots in the environment chamber for three weeks, watering as necessary.

2). **Florigen Movement from the Leaf:** obtain six more *Pharbitus nil* seedling pots. Label the pots "florigan movement," numbering them one through six, and place them in the darkroom. Organize **seven more** students to return the pots to the long-day conditions in the environment chamber after sixteen hours of dark. Additionally, remove the cotyledons of all plants in the following sequence.

Pot #1 — remove the cotyledons after 12 hours of dark (leave in dark for four more hours.)

Person Responsible ______________________________

All Pots — remove from the dark after 16 hours and put in the environment chamber with timer set at 16 hours light, 8 hours dark.

Person Responsible ______________________________

Pot #2 — remove cotyledons 2 hours after end of the continuous dark period.

Person Responsible ______________________________

Pot #3 — Remove cotyledons 4 hours after end of the continuous dark period.

Person Responsible ______________________________

Pot #4 — remove cotyledons 8 hours after end of the continuous dark period.

Person Responsible ____________________

Pot #5 — remove cotyledons 12 hours after end of the continuous dark period.

Person Responsible ____________________

Pot #6 — remove cotyledons 20 hours after end of the continuous dark period.

Person Responsible ____________________

Leave pots in the environment chamber for three weeks, watering as necessary.

Procedure: Hour 8

Students need the following: set ups from hour seven step B (light, gibberellin and growth) and hour one step E (control of bud dormancy), ruler, razor, paper, lanolin, 1% IAA in lanolin, and 1% GA in lanolin.

Students need access to the greenhouse, as well as dark, red light, and fluorescent light enclosures.

During this laboratory hour you will observe again the effects of light on plant growth, as well as the effect of gibberellin on plant growth. You also will continue the experiment begun during hour one on the control of bud dormancy.

There should be seven days between hours seven and eight of this laboratory, or fifteen days between the first hour of this laboratory and the eighth hour. Plants need this time to grow.

Steps

A. **Light, gibberellin, and growth:** examine the six pea seedling pots begun in hour one, placed in the dark, fluorescent light, and red light enclosures, and manipulated during hour seven of this laboratory. When examining the plants in the darkroom, use a flashlight covered with green cellophane to produce a dim green light. For each plant, note the length of the stem, the number of nodes, the number of leaves, the size of the largest leaf, and the shape of the leaf apex.

28. Record your findings in the figure thirteen chart below (w/o refers to the plants to which ethanol without (w/o) gibberellin was applied).

Growth	Dark				Red				Fluorescent			
	Alaska		Dwarf		Alaska		Dwarf		Alaska		Dwarf	
	GA	w/o	GA	w/o	GA	w/o	GA	w/o	GA	w/o	GA	w/o
Average Stem Length												
Average Node Number												
Average Leaf Number												
Largest Leaf Size												
Shape Leaf Apex												

Figure 13

29. What effects did the fluorescent light, dim red light, and dark have on plant growth? ____________

30. What differences did you notice in the way those plants treated with GA reacted to fluorescent, dim red, and no light?

B. Return the plants to their original lighting conditions for another five days. Water them as required.

C. **Control of bud dormancy:** retrieve from the greenhouse the four pots with Alaska peas planted during hour one step E. Decapitate eight plants in each pot half-way up the sixth internode (leave any additional plants so as not to disturb roots). Label each pot #1 through #4 and affix your name. Cover stumps of the decapitated plants with the following chemicals.

Pot #1 — lanolin.

Pot #2 — 1% IAA in lanolin.

Pot #3 — 1% GA in lanolin.

Pot #4 — Both 1% IAA in lanolin and 1% GA in lanolin.

Return the pots to the greenhouse and allow them to continue growing for another five days. Water as necessary.

Procedure: Hour 9

Students need the following: set ups from hour eight, steps A and C, and a ruler.

Students need access to the greenhouse and the dark, red light, and fluorescent light enclosures.

During this hour you will observe again the effects of light on plant growth, and also the effect of gibberellin on plant growth. You also will conclude the experiment begun in hour one and continued in hour eight on control of bud dormancy.

There should be five days between hours eight and nine of this laboratory, or twenty days between the first hour of this laboratory and the ninth hour. Plants need this time to grow.

Steps

A. **Light, gibberellin, and growth:** examine the six pea seedling pots begun in hour one, placed in the dark, fluorescent light, and red light rooms, and manipulated during hours seven and eight. When examining the plants in the darkroom, use a flashlight covered with green cellophane to produce a dim green light. For each plant, note the length of the stem, the number of nodes, the number of leaves, the size of the largest leaf, and the shape of the leaf apex.

31. Enter your findings in the figure fourteen chart below.

Growth	Dark				Red				Fluorescent			
	Alaska		Dwarf		Alaska		Dwarf		Alaska		Dwarf	
	GA	w/o	GA	w/o	GA	w/o	GA	w/o	GA	w/o	GA	w/o
Average Stem Length												
Average Node Number												
Average Leaf Number												
Largest Leaf Size												
Shape Leaf Apex												

Figure 14

32. What effects did fluorescent light, dim red light, and dark have on plant growth?

33. What differences did you notice in the way those plants treated with GA reacted to fluorescent, dim red, and no light?

B. **Control of bud dormancy:** retrieve from the greenhouse the four pots with Alaska peas decapitated and treated with chemicals during hour eight step C. Measure in millimeters the lateral buds on all plants.

34. Record the length of all lateral buds on all plants in the chart, figure fifteen, as follows:

Lateral Buds:	**#1**	**#2**	**#3**	**#4**	**#5**	**Average**
Plants from Pot #1 (treated with lanolin):						
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
					Total Average	
Plants from Pot #2 (treated with 1% IAA in lanolin):						
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
					Total Average	

(Continued)

Lateral Buds:	#1	#2	#3	#4	#5	Average
Plants from Pot #3 (treated with 1% GA in lanolin):						
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
				Total Average		
Plants from Pot #4 (treated with 1% IAA and 1% GA in lanolin):						
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
				Total Average		

Figure 15

35. From these results, explain the role of GA in apical dominance.

36. From these results, explain the role of IAA in apical dominance.

37. From these results, explain the cumulative effects of GA and IAA together on apical dominance.

Procedure: Hour 10

Students need the following: set up from hour seven step C (morning glories).

During this laboratory hour you will observe the results of previous manipulations of *Pharbitus nil*. You will determine the critical night length, and the rate of movement of florigen from the leaves.

Hour ten cannot be run until twenty-eight days after hour one, or twenty-one days after hour seven, when the morning glories were first manipulated.

Steps

A. **Critical night length:** study the seven pots subjected to varying hours of darkness. Count the number of flower buds on each plant.

38. In the chart, figure sixteen, record the number of flower buds.

Time Plants In Dark	Average Bud Number Per Plant
8 Hours	
9 Hours	
10 Hours	
11 Hours	
12 Hours	
14 Hours	
16 Hours	

Figure 16

39. What is the critical night length for morning glories? ____________________

40. If a plant's flowering were unaffected by variations in hours of dark, and you wanted to test to see if it were affected by hours of light, how could you set up an experiment to test critical day length?

B. **The Rate of Florigen Movement from the Leaf:** study the six pots of *Pharbitus nil* from which cotyledons were removed at varying times, after having been subjected to darkness. Determine the number of flower buds per plant for each treatment.

41. In the chart, figure seventeen, record the average number of flower buds per plant per treatment.

Cotyledons Removed	Average Bud Number
4 Hours Before Completion of 16 Hours Placement in Dark	
2 Hours After Movement to Environmental Chamber	
4 Hours After	
8 Hours After	
12 Hours After	
20 Hours After	

Figure 17

42. What is the rate of florigen transport from morning glory leaves? ____________

43. In what ways might florigen be used in the horticultural industry? ____________

Resources

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Erickson, Ralph O. and Wendy Kuhn Silk. "The Kinematics of Plant Growth," *Scientific American*, May 1980.

Juniper, Barrie E. "Geotropism," *Annual Review of Plant Physiology*, Vol. 27, 1976.

Ray, Peter M. *The Living Plant*, 2nd Ed. Holt, Rinehart and Winston: New York, 1972.

Rosenthal, Gerald. "The Chemical Defenses of Higher Plants," *Scientific American*, January 1986.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

- auxin
- critical light length
- florigen
- gibberellin
- indole acetic acid
- kinetin

Review

28. Plant Growth

Name

Date

Essay

1. How do auxins, gibberellins, and cytokinens affect lettuce seed germination?

2. Define short-day, long-day, and day-neutral plants. How does florigen interact with gibberellin in the flowering response? How does this connect with day/night length responses?

3. What role do auxins play in phototropism and apical dominance?

Answers Found: throughout entire laboratory.

29. Laboratory

Plant and Water Relationships

When you have completed this laboratory you should be able to:

1. Determine by a graded solution method the water potential of potato tissue.
2. Determine the rate of water conduction/transport per hour in a herbaceous plant.
3. Determine the water holding capacity of four soil types.
4. Determine the effect of temperature on the rate and total imbibition of water by kelp.
5. Determine the effect of soil water percentage on seed germination.
6. Determine the path of water transport in a woody plant.

Purpose

All living organisms require water. Indeed, they are composed mostly of water, and maintenance of internal water levels is essential to life. To properly study living plants, water relationships must be fully known. This laboratory introduces the student to several aspects of plant/water relationships through studies to be made in six mini-experiments.

Water Potential Determination

Under natural conditions, practically all water absorption by rooted plants takes place through the root hairs. Water diffuses into the root hairs, and other epidermal cells, as a result of a diffusion pressure deficit gradient. As long as the diffusion pressure deficit of the root cells' sap is greater than that of the soil solution, water will enter the cell. Any increase in the concentration of minerals or decrease in turgor pressure will increase the diffusion pressure deficit of the cell sap, thereby increasing the uptake of soil water. We therefore can say that most water absorption occurs through passive osmotic mechanisms.

The student will determine the water potential of potato tissue during the first experiment of this laboratory. Water potential of tissue is dependent upon the amount of solute concentrated in water surrounding the tissue. Solute concentration is comparable to osmotic pressure. When graphed, the student will be able to find an equilibrium where water is neither gained nor lost and to determine the corresponding osmotic pressure value which indicates the water holding potential of the tissue.

Soil Water Percentage and Seed Germination

During the second experiment the amount of soil water required to effectively trigger corn seed germination is measured. As noted in laboratory twenty-eight, there are many factors which might trigger or otherwise influence seed germination, not the least of which is seed type. Light requirements, temperature requirements, mechanical resistance of the seed coat, seed coat permeability properties, and hormonal controls are some of these factors. This experiment gives the student the ability to specifically test one mechanical germination factor, soil water requirements.

Imbibition and Temperature

The effects of temperature upon imbibition rate and total absorption are studied during the third experiment. This experiment is a graphic reminder that all of the plant/water experiments in this laboratory would yield different values if performed at temperatures other than room temperature.

Water Holding Capacity

Different soils have different water holding capacities. The water holding capacity of soils depends on many factors, such as the physical size and structure of soil particles. Other factors include the diffusion pressure of water in the soil. The more solutes in the water, the lower the diffusion pressure. Diffusion pressure can be increased or decreased by atmospheric pressure. Weather conditions and altitude thus are factors which also affect the water holding capacity of soil.

When a soil type holds the maximum amount of water it can without being waterlogged, it is considered to be at field capacity. When a soil is holding so little water that a plant can no longer extract enough water through its root hairs to maintain life, the soil is considered to have reached its permanent wilting percentage, or wilting coefficient. A plant reaches a state of permanent wilt when it can no longer regain turgor when watered.

The term "permanent wilting percentage" is a rather unfortunate choice of words because the term refers to water held by a soil type under specific conditions, and yet it defines that soil condition by reference to plant growth. The permanent wilting percentage of soil does vary according to the type of plant growing on it, although these species dependent variations account for only about ten percent of the permanent wilting percentage of any given soil.

The fourth experiment tests the water holding capacities of four soil types. An experiment to test the permanent wilting percentage of soil is introduced as an optional activity at the end of this laboratory.

Water Movement Through a Stem

The timed movement of water through an herbaceous stem is studied during the fifth experiment. The actual path of water movement in a woody stem is studied during the sixth experiment. An experiment to demonstrate transpirational pull, or how water can be carried to the tops of the tallest trees by simple osmotic mechanisms, is given as an optional activity at the end of this laboratory.

Pre-lab

Supplies needed:

Equipment

10 125 ml. wide erlenmeyer flasks
10 stoppers to fit flasks
tray for 10 flasks
600 ml. beaker
2 1000 ml. erlenmeyer flasks
dowel
refrigerator
incubator
drying oven
500 ml. graduated cylinder
5 ml. graduated cylinder
2 1-holed rubber stoppers to fit 1000 ml. flasks
4 #300 tin cans with bottoms perforated (14 ozs., or @ 400 ml.)
1 cm. diameter cork borer
knife
balance or scale
ruler
4 15-cm. (6-inch) plastic pots
6 culture dishes
4 petri dishes
pan to hold 4 #300 tin cans
photoflood light
screen to hold tin cans

Materials

500 ml. 1M sucrose solution
1 large potato
soft wax
6,400 g. air dry greenhouse loam
aluminum foil
12 g. dry kelp (kombu)
4 soil types
2 uniform branches of an evergreen, such as hemlock or cedar
1 stem *Impatiens* (garden balsam) or celery stalk
filter paper
7 cm. diameter filter paper
100 ml. eosin solution
plastic wrap
2 liters distilled water
40 2-hour soaked corn seeds

Special Preparations

1) To assure that there are enough tin cans for the entire class, start collection well before the actual laboratory.

2) 1M sucrose is made by dissolving 342 g. sucrose in distilled water, and adding more distilled water to make 1 liter. Mix enough to supply 500 ml. to each laboratory group.

3) Malleable grafting wax is a soft wax made by slowly heating 4 parts rosin, 2 parts beeswax, and 1 part tallow until melted and well mixed. Cool by pouring mixture into cool water, and then shape it into balls with the fingers.

4) Eosin solution: mix 0.5% eosin in distilled water.

5) Dried kelp is available through any oriental grocery store, or biological supply houses.

6) At least three hours prior to hour three of this laboratory, equilibrate the six culture dishes and distilled water for all laboratory groups at the temperatures called for in the experiment. The exact temperatures can vary slightly from those listed for the experiment. The lowest temperature can be achieved by setting the culture dishes and distilled water in a refrigerator, the second simply by leaving the culture dishes and water at room temperature, and the third by placing them in an incubator.

7) Test soil types from your locality and select four with meaningful differences in water holding capacity.

Time Required

The plant and water relationships laboratory requires six full classroom hours and one partial additional hour, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the following: 500 ml. 1M sucrose solution, graduated cylinders, 10 125 ml. wide erlenmeyer flasks with stoppers, 1 cm. diameter cork borer, 1 large potato, filter paper, scale or balance, tray, refrigerator, 4 #300 tin cans with the bottoms perforated, 4 sheets 7 cm. diameter filter paper, 4 soil types, 4 petri dishes, pan, and water.

In this laboratory you will set up both an experiment to determine the water holding potential of potato tissue, as well as an experiment to test the water holding capacity of four soil types.

Steps

A. **Water Potential:** using 500 ml. stock 1M sucrose solution and serial dilution techniques, prepare 100 ml. each of the ten diluted solutions listed below, placing each dilution in a 125 ml. wide erlenmeyer flask. The serial dilution technique used in laboratory #9 was a halving technique, whereas this serial dilution procedure requires use of the following equation: initial molarity times initial volume equals final molarity times final volume ($M_i \times V_i = M_f \times V_f$). Label each flask with the molar concentration, your name, and today's date.

Sucrose concentration in moles per liter (M/l):

#1 - 0.60	#6 - 0.35
#2 - 0.55	#7 - 0.30
#3 - 0.50	#8 - 0.25
#4 - 0.45	#9 - 0.20
#5 - 0.40	#10 - 0.15

B. Drill ten cylinders from a large potato with a cork borer exactly one cm. in diameter. Using a knife and ruler, trim each cylinder to a length of four cm., eliminating all potato skin in the process. Blot the cylinders by rolling them on filter paper, weigh each one to the nearest centigram, and place one cylinder in each of the ten flasks filled with sucrose solution during step A. Label each flask with the weight of the potato cylinder placed in it. Stopper the bottles, place them on a tray, and put them in the refrigerator for at least twenty-four hours.

1. Record the weight of the potato cylinders in figure one.

Flask	Grams Potato	Flask	Grams Potato
#1 - 0.60		#6 - 0.35	
#2 - 0.55		#7 - 0.30	
#3 - 0.50		#8 - 0.25	
#4 - 0.45		#9 - 0.20	
#5 - 0.40		#10 - 0.15	

Figure 1

C. **Water Holding Capacity:** clean and remove old labels from four #300 tin cans which have the bottoms perforated. Place a seven cm. filter paper in the bottom of each can, then weigh each can with filter paper to the nearest 0.05 g. Label the can with its weight, your name, and today's date.

D. Fill one can to a depth of 4 cm. with one of the four soil types available. Tap gently to settle the soil and obtain a true 4 cm. depth. Note on the can the type of soil it contains. Cover the can with an inverted petri dish, and set it in a pan containing two or three cm. water. Repeat the process for the other three cans and soil types. Leave them for at least twenty four hours.

Procedure: Hour 2

Students need the following: set ups from hour one, screen, scale, filter paper, 6,400 g. air dry greenhouse loam, aluminum foil, plastic wrap, 4 8-cm. plastic pots, distilled water, 500 ml. graduated cylinder, dowel, ruler, and 40 2-hour soaked corn seeds.

During this laboratory you will conclude the experiment on water holding potential in potato tissue, continue the experiment on water holding capacity of soil, and set up an experiment on the effect of soil water levels on corn seed germination.

Steps

A. Remove the potato cylinders from the sucrose solution concentrations **one at a time**. Blot them carefully on filter paper, and weigh at once.

2. Record your results in the figure two chart below. The osmotic potential is listed in atmospheres. Weights should be listed in grams.

Sucrose Conc. (M/L)	Osmotic Potential	Initial Weight	Final Weight	Gain or Loss
0.60	17.8			
0.55	16.0			
0.50	14.3			
0.45	12.7			
0.40	11.1			
0.35	9.6			
0.30	8.1			
0.25	6.7			
0.20	5.3			
0.15	4.0			

Figure 2

3. Plot the gain or loss of weight against sucrose concentration in the figure three graph on page 440. Draw a straight line through the graph where there is neither weight gain nor loss. Where the curve crosses this line yields a figure for the water potential of potato tissue in atmospheres (refer to figure two).

4. What is the water potential of potato tissue in atmospheres? ______________________

5. Why is information concerning water potential and osmotic pressure useful? ______________________

Figure 3

6. Describe another method to obtain this data. ______________________________

____

____

____

____

B. Retrieve the laboratory set up on water holding capacity. Remove the tin cans from the pan of water and place them on a screen to drain for two days (keep petri dish lids in place).

C. **Effect of Soil Water Percentage on Seed Germination:** weigh out four 1,600 g. lots of air dry greenhouse loam. Place each lot on a large piece of plastic wrap. Thoroughly mix (by rolling and moving the plastic wrap) each soil lot with the following amounts of distilled water:

Soil lot #1 — add 400 ml. distilled water.
Soil lot #2 — add 200 ml. distilled water.
Soil lot #3 — add 100 ml. distilled water.
Soil lot #4 — add 50 ml. distilled water.

D. Put each soil lot into an 8 cm. diameter plastic pot, and settle them uniformly by tapping the pot on your work bench. Label each pot both by number and by the amount of distilled water added, as well as your name and today's date. Punch ten uniformly spaced holes in the top of the soil in each pot. Make sure the holes are exactly 2.5 cm. deep. Use a dowel to do this by placing a measured line around the dowel 2.5 cm. from the tip and using the dowel as a depth gauge.

E. Dry by blotting with filter paper forty 2-hour soaked corn seeds. Select the corn seeds for uniformity of size. Drop one seed, tip down, in each hole, and cover each seed uniformly with soil. Completely cover each pot with aluminum foil, and set aside for two days.

Procedure: Hour 3

Students need the following: 12 g. dry kelp, scale, filter paper, graduated cylinder, and 6 culture dishes and distilled water equilibrated at approximately 5, 15, and 25° C. (see item six of special preparations).

During this laboratory hour you will begin to determine the effect of temperature upon the rate and volume of water imbibition by dry kelp. **These operations on kelp must be completed within 48 hours. Therefore, the water imbibition experiment must be scheduled so that weekends and holidays do not intervene.**

Steps

A. Label two culture dishes equilibrated at 5° C. with the temperature, your name, and today's date, and with the numbers one and two. Fill each dish with 100 ml. distilled water which is also at 5° C. Perform similar procedures on two culture dishes equilibrated at 15° C., or room temperature, and also on two dishes equilibrated at 25° C. You should have a total of six labelled dishes numbered one through six. After each operation, return the dishes and water to their previous temperature controlled storage areas.

B. Weigh to the nearest 0.01 g. six similar pieces of dry kelp each weighing between one and two grams. At zero hour, place one piece of kelp into each culture dish. It is better if two or three people coordinate to complete this and the following tasks in the shortest possible time. Label the outside of the culture dish with the exact weight of the kelp placed in it. Wait **exactly** five minutes from zero hour.

C. After five minutes, quickly remove each piece of kelp, and blot vigorously with filter paper. Weigh quickly on a scale to the nearest 0.01 gram, and then return to the proper culture dish.

7. Record your weight data in the chart, figure four, in the appropriate time column.

Dish	°C.	Dry Kelp (g.)	Time in Minutes from Zero Hour					
			5	15	35	50	24 hrs.	48 hrs.
#1								
#2								
#3								
#4								
#5								
#6								

Figure 4

D. Fifteen minutes from zero hour again remove each piece of kelp, blot vigorously with filter paper, weigh quickly on a scale to the nearest 0.01 gram, and return it to the proper culture dish. The dishes in turn should be returned to the appropriate temperature controlled area.

8. Record your data in the chart, figure four.

E. Repeat step D at 35 and 50 minutes past zero hour.

9. Record your data in the chart, figure four.

F. Leave your experimental set up for twenty-four hours.

Procedure: Hour 4

Students need the following: laboratory set ups from hour two steps B and E (tin cans with moist soil and potted corn seeds) and hour three step F (kelp soaking in water), filter paper, scale, and drying oven.

During this laboratory hour you will continue the experiments on the effect of temperature on the rate and volume of water imbibition by kelp, soil water holding capacities, and the effect of soil water percentage on seed germination.

Steps

A. **Kelp Water Imbibition:** repeat hour three, step D procedure.

10. Record your data in the chart, figure four, in the 24 hour column.

B. **Water Holding Capacity:** Weigh the drained soil and can to the nearest 0.1 gram.

11. Record the soil type as labeled on the respective cans in the space provided below.

#1 ____________________

#2 ____________________

#3 ____________________

#4 ____________________

12. Record the weight of each can with filter paper, as written on the outside at the beginning of the experiment, and also the soil filled cans after excess water has been drained off, entering these weights in figure five below. Enter only those two weights; other data will be entered and explained subsequently.

Weight Can with paper	Weight Drained Soil	Weight Dried Soil	Net Water Loss	Water Holding Capacity
#1				
#2				
#3				
#4				

Figure 5

C. Place the cans in a drying oven for one to two days.

D. **Soil Water Percentage and Seed Germination:** observe the set up from hour two step E (potted corn seeds). Count the number of shoots emerged from each pot.

13. Record the number of shoots emerged in the chart, figure six, under day two column.

Pot	Water ml.	% Moisture	Shoots Emerged		
			2 days	4 days	7 days
#1	400 ml.	25%			
#2	200 ml.	13%			
#3	100 ml.	6%			
#4	50 ml.	3%			

Figure 6

Figure 7

E. Again set aside the potted corn seedlings for two days, with the pots completely covered by aluminum foil.

Procedure: Hour 5

Students need the following: set ups from hour four steps A and C (soaked kelp and dried soil), scale, filter paper, and ruler.

During this laboratory hour you will conclude the experiment on the effects of temperature on the rate and volume of water imbibition by kelp and the experiment on water holding capacities of four soil types.

A. **Kelp Imbibition:** repeat hour three, step D procedure.

14. Record your data in the chart, figure four, under 48 hour column (chart on page 442).

15. Plot water uptake by dry kelp, in figure seven, by placing time data on the vertical axis and grams water increase per gram kelp on the horizontal axis. Plot curves for each of the three temperatures on the same graph (graph on page 444).

16. Calculate the difference in the rate of absorption for each ten degree change. Record your data in the figure eight chart below.

10° C. Change	Absorption Rate (g. Water/g. Kelp/Minute)
5 to 15°C.	
15 to 25°C.	
Difference	

Figure 8

17. Can you distinguish between the rate of water imbibition in kelp at different temperatures and total imbibition over a 48 hour period? How?

B. **Water Holding Capacity:** Weigh each dried soil and can to the nearest 0.1 gram.

18. Record the weight of each oven dried can with soil and paper in the chart, figure five (page 443). The cans can now be discarded.

19. Find the difference between drained soil weight and dry soil weight. This net difference is due to evaporated water. Record this net water loss in the chart, figure five.

20. Compute the water holding capacity of each soil type. The water holding capacity can be determined by dividing the total net water loss in grams by the total dry soil weight in grams.

21. Record the water holding capacity for each soil type in the chart, figure five.

Procedure: Hour 6

Students need the following: set up from hour four step D (potted corn seeds), stem of garden balsam *(Impatiens)* or celery stalk, 600 ml. beaker, approximately 150 ml. eosin solution, photoflood light, ruler, knife, 2 fresh branches of a woody evergreen, wax, scale, and 2 1000-ml. erlenmeyer flasks with one holed stoppers.

During this laboratory hour you will continue the experiment on the effect of soil water percentage on seed germination. You also will test the rate of water movement through an herbaceous stem. Additionally, you will set up an experiment to test the path of water movement through a woody stem.

Steps

A. Soil **Water Percentage and Seed Germination**: observe the potted corn seeds last studied during hour four, and count the numbr of emerged shoots.

22. Record the number of emerged shoots in the chart, figure six (page 443), under four day column.

B. Return the potted corn seedlings to storage, again completely covered by aluminum foil. Leave for two or three more days.

C. **Rate of Water Movement**: place a sturdy stem of garden balsam *(Impatiens)* or a fresh celery leaf stalk into a 600 ml. beaker containing eosin solution, an organic dye, to a depth of about two centimeters. Illuminate with a photoflood light. Time the experiment. **You may wish to begin step D while waiting for results in this step.** When there is any indication of staining in any leaf, quickly measure the distance the dye has travelled. Also observe the time the experiment has taken.

23. What type of plant stalk did you use? ____________________

24. What was the elapsed time of your experiment? ____________________

25. What was the distance travelled by the eosin solution? ____________________

26. What is the rate of water movement in your herbaceous stem? Conduction is measured in centimeters per hour. ____________________

D. **Path of Water Movement**: obtain two uniform branches of a woody coniferous evergreen. Make fresh cuts at the base end of both branches. Plug the xylem on one with soft wax, leaving the phloem open. Plug the phloem on the other, leaving the xylem open. (You may wish to review gymnosperm branch structure in laboratory 21).

E. Insert the base ends of the stems into rubber stoppers. You may have to make the stopper holes larger in order to accommodate the stems. Place a stopper with one evergreen stem into a 1000 ml. erlenmeyer flask filled with 500 ml. water. Label each flask with your name and today's date, and whether the xylem or phloem was plugged. Weigh both complete set ups, record the weight on the flask, and leave for one week. (You will re-weigh after a week and compare the water loss by the stems).

27. Record the initial weights in the chart, figure nine, on the next page.

	Weights (grams)	
	Phloem Plugged	**Xylem Plugged**
Date Initial		
Date Final		
Water Loss		

Figure 9

Procedure: Partial Hours 7 and 8

Students need the following: set ups from hour six step B (potted corn seed) and C (evergreen stem in flasks), and a scale.

During these partial laboratory hours you will conclude the experiment on the effect of soil water percentage on seed germination, and determine the path of water movement in woody stems.

Timing: do step A two or three days after laboratory hour six. Allow five minutes only for this step. Do step B one week after laboratory hour six. Allow approximately fifteen minutes for this step.

Steps

A. **Soil Water Percentage and Seed Germination:** observe your set up from hour six step B (potted corn seeds after four days). Count the number of shoots emerged from each pot.

28. Record the number of shoots emerged in the chart, figure six (page 443).

29. Plot percent emergence of corn seedlings as a function of time against soil moisture percentages on the graph, figure ten, on the next page.

30. What is the critical water percentage for good corn seed germination? ____________________

____

B. **Path of Water Movement:** reweigh the two evergreen branch set ups, and note the water loss through each branch.

31. Record the new weights in figure nine. Determine water loss in the two branches by subtracting final weights from initial weights.

Figure 10

32. Compare water loss through each of the branches. Which tracheid system absorbs water and which system loses water? How can you tell from this information the paths of water movement in plants?

Optional Activities

Students may wish to explore other aspects of plant and water relationships. Listed below are two experiments, one on permanent wilting percentage and the other on plant transpiration. Students can submit their results to the instructor in a formal laboratory report.

1) **Permanent Wilting Percentage:** six to eight weeks prior to this laboratory, four tomato seedlings should be planted in each of four pots with good garden soil, and with a 5-6 cm. glass cylinder fitted around the main stem of each seedling.

When you are ready to begin the experiment, seal the lower end of the cylinder with soft wax, and fill the cylinder to within one centimeter of the top with the soil you wish to test. This soil should be a type which has already had its water holding capacity determined, as during this laboratory. Moisten by adding water drop by drop. Do not waterlog. Return the plants to the greenhouse. Water the main plants normally, but **do not** water the dirt in the cylinder.

After six weeks remove the soil from the cylinder, separating out the roots which have grown into it from the stem. An easy way to do this is to slice off the entire tomato stem both above and below the cylinder, remove the stem section and roots carefully so as to minimize soil loss, and then empty the soil from the cylinder. Weigh the soil without delay, place it in a drying oven for two days, and weigh again. Your data may be easily read from a chart such as the following, figure eleven. Soil type and its water holding capacity have been previously determined. Tare weight is what you measure after six weeks of drought. Although the soil still holds some water, the plant is no longer able to withdraw that residue. The soil at that point has reached its permanent wilting percentage.

Soil Type	1. Water Holding Capacity	2. Tare Weight	3. Weight Loss #1 - #2	4. Oven Dry Weight	6. PWP #3 ÷ #4 × 100	6. Available Water WHC-PWP

Figure 11

2) **Transpirational Pull:** fill a small bore capillary tube which is 100 cm. or more in length with recently boiled, distilled water. Attach a woody shoot of *Chamaecyparis* or *Thuja* to one end of the capillary tube with a length of heavy rubber tubing, and place the other end of the glass tube into a test tube of water. Great care must be taken to avoid introducing air bubbles into the system.

Pour a small amount of clean mercury into the test tube, so that mercury covers the end of the capillary tube. Observe this set up periodically, and record the maximum height reached by the mercury. If more than 76 cm. was recorded, how would you interpret such a result?

Instead of using a woody evergreen twig, you can perform the same experiment by demonstrating transpirational pull using a porus clay cup in place of the twig.

Resources

Carlquist, Sherwin. "Types of Cambial Activity and Wood Anatomy of *Stylidium (Stylidadidaceae)*," *American Journal of Botany*, June 1981.

Heslop-Harrison, Yolande. "Carnivorous Plants," *Scientific American*, February 1978.

Triplett, G. B. and D. M. Van Doren. "Agriculture Without Tillage," *Scientific American*, January 1977.

Terminology

Students should understand the following terms and concepts prior to taking the unit review.

permanent wilting percentage
water holding capacity

Review
29. Plant and Water Relationships

Name ______________________________

Date ______________________________

Short answer

1. Define the term "water holding capacity". ______________________________

2. Define the term "permanent wilting percentage". ______________________________

3. Distinguish between the paths of water movement in xylem and phloem in a gymnosperm. __________

4. How does temperature affect imbibition in a plant? ______

5. How does soil water content affect germination of seeds? ______

Answers Found: throughout entire laboratory.

30. Laboratory

Mineral Absorption, Use, and Translocation in Plants

When you have completed this laboratory you should be able to:

1. Determine the minimum mineral requirements of a plant.
2. Determine the absorption of iron by barley roots.
3. Determine the effects of temperature and a competing ion on mineral absorption rate.
4. Trace the translocation of Fe^{59} in a plant.

Purpose

Minerals are absorbed by plants during the imbibition and transpiration of water. Minerals, mostly in the form of salts, accumulate in the xylem ducts of the root and translocate to the shoot. Once in the shoot, they are distributed and redistributed throughout the entire plant. The plant uses different minerals in different ways. It is able to move minerals to areas of greater need or to save minerals prior to leaf drop by moving the minerals to other areas of the plant. Some minerals are highly mobile while others are not. Different plant species have different mineral requirements, or utilize similar amounts of the same minerals in different ways.

Essential Mineral Elements

Macro-nutrients

Carbon, hydrogen, and oxygen are the three elements present in all carbohydrates, fats, proteins, and other organic components of living organisms. Plants obtain them from atmospheric carbon dioxide and from water.

Nitrogen is absorbed by plants as nitrate or ammonium ions derived from the decomposition of organic matter in the soil, and by other means. Nitrogen functions as a primary constituent of proteins, as well as of other plant parts such as chlorophyll.

All remaining plant nutrients are obtained from soil minerals.

For example, phosphorus, obtained from the soil, functions as a constituent of some fatty substances and of special proteins found in cell nuclei and chromosomes. It also is a component of starch synthesizing systems in many plants and a component of the adenylic acid system which stores chemical energy in cells for later release.

Sulfur serves as a constituent of many proteins, including certain enzymes.

Calcium is present in calcium pectate, which is the principal component of the middle lamella of leaves and generally maintains the differential permeability of cellular membranes. It also promotes the activity of certain enzymes, such as amylase.

Magnesium is a basic constituent of chlorophyll, and some magnesium also promotes the activity of certain enzymes, especially those which function in carbohydrate transformations.

Potassium functions in the synthesis of proteins and in the translocation of carbohydrates.

Iron functions in the synthesis of chlorophyll and as a constituent of several important oxidative enzymes. Iron actually might better be called a micro-nutrient, because it is required by the plant in amounts similar to that of micro-nutrients.

Micro-nutrients

Boron functions in the synthesis of proteins and pectins in the plant.

Manganese is required in the synthesis of chlorophyll and also in the synthesis and digestion of fats and oils.

Zinc functions in the synthesis of the auxin IAA, indole acetic acid, and it also is a constituent of the enzyme carbonic anhydrase.

Copper is a constituent of enzymes (the polyphenol oxidases) which cause the browning reaction on cut surfaces of fruits and vegetables. Incidentally, you exploited the browning reaction in laboratory seven on enzyme activity, when you measured light transmittance and absorbance with the spectrophotometer. (Researchers do not yet know of other uses of copper in plants.)

Molybdenum is active in transformations of nitrogen, as in nitrogen fixation by bacteria and nitrate reduction by higher plants.

Chlorine is considered to be a micro-nutrient, but its function is as yet unknown. Its presence in plants was discovered only in 1960.

Additionally, some plants require yet other elements in trace amounts. For example, diatoms require silicon, some algae require vanadium, and galium is required by some fungi. Sodium stimulates the growth of some plants, such as sugar beets, and most desert plants. There is evidence that nickel, cobalt, aluminum and titanium also are required by some plants; but why, how, and in what amounts has yet to be determined.

Radioactive Tracers

Until the development of radioactive tracing techniques plant physiologists encountered great difficulties determining which vascular tissues provide passage for mineral salts from one area of the plant to another. Since the introduction of radioactive tracers, several different pathways for the translocation of salts have been discovered. For example, mineral salts move in the xylem, in the phloem, laterally between these two tissues, and outward from the leaf.

Mineral Absorption, Utilization, and Translocation

This laboratory is designed as three introductory mini-experiments in the determination of minimum mineral requirements of a plant, the monitoring of plant mineral uptake, and the tracing of the translocation of a mineral throughout a plant.

Mineral Deficiency Culture

The first experiment is designed to explore the mineral nutritional requirements of the mold, *Aspergillus niger*. The mold will be grown both in complete nutrient solution and in solutions deficient in different elements. As an optional activity at the end of this laboratory, directions are given for the growth of tomato seedlings without soil, some in complete nutrient baths, and others with deficient nutrient baths.

Mineral Absorption

The second experiment involves testing barley roots for mineral uptake over a period of time and determining how temperature affects that uptake. Mineral absorption also is tested to determine how the presence of a competing ion affects uptake. Uptake is traced and monitored by adding a radioactive tracer and counting the absorbed radioactive substances on a scintillation well counter.

Iron Translocation

The third experiment involves tracing the circulation and reutilization of a mineral once it has been absorbed into the plant. Different minerals are utilized in different ways. This experiment traces the path of iron in a plant by using a radioactive tracer, Fe^{59}. The plant is blotted and then placed against film which is allowed to develop for two to three days in a darkroom. In essence, the plant photographs itself with absorbed radioactivity. The result is called an autoradiograph.

Radioactive Tracing in Studies of Translocation

Minerals generally are taken up in the transpiration stream and exported to the leaves, with excess quantities of minerals relocated downward in the phloem. Minerals can be transported laterally into xylem tissue where they can again be translocated upwards to repeat the cycle until completely used by the plant. Elements such as nitrogen, potassium, and phosphorus move readily within this circuit, recycling many times. Because phosphorus is highly mobile and also available as a radioactive isotope, there are many experiments in which an autoradiograph of radioactive phosphorus in plants can be taken.

Radioactive calcium is taken up in a fashion similar to phosphorus, nitrogen, and potassium. However, calcium is immobile in the phloem, and once delivered by the transpiration stream, it remains stationary in the phloem until utilized.

Radioactive sulfur also is mobile in plants, but because of its rapid uptake into metabolic compounds, it does not circulate in the plant like phosphorus, nitrogen, and potassium. When sulfur is translocated, it rapidly becomes concentrated in the plant's younger leaves. Mature leaves lose their sulfur content to younger, faster growing leaves. All this occurs within twenty-four hours of uptake. Sulfur is a constituent of proteins, and protein synthesis occurs to a much greater extent in young leaves. Therefore, sulfur circulating within a plant is captured metabolically by sites of protein synthesis within the plant. In this fashion sulfur rapidly is rendered immobile in plants.

Radioactive iron tracing within the tissues of a plant yields information on the uptake and subsequent mobility of iron. Iron mobility is dependent primarily upon iron concentration in plant tissues, but it is dependent secondarily upon phosphorus availability and on pH. When iron concentration is low in plant tissue, uptake and translocation of iron into the phloem is highest. As iron increases in the tissues, the mobility of iron decreases. A pH of four gives high iron mobility. This mobility decreases as the pH increases to seven. Low phosphorus availability increases iron mobility. As phosphorus concentrations increase, iron mobility decreases.

Radioactive iron is used in the third experiment employing autoradiography, because iron is readily taken up and moved about within plants. The many variations possible in iron uptake make it possible to experiment further with the absorption, utilization, and translocation factors influencing Fe^{59} uptake. Students are urged to continue these experiments on their own.

Pre-lab

Supplies needed:

Equipment

16 culture jars with caps
scale or balance
scintillation well counter
550° C. furnace
incubator
plant press
glass stirring rod
bunsen burner or open flame source
10 ml. graduated cylinder
scissors
16 watchglasses
dessicator
clothesline
autoclave, or pressure cooker and stove
15x140 mm. counting tube with screw cap
inoculating loop

12 evaporating dishes
drying oven
refrigerator
photographic darkroom
100 ml. beaker
4 rinse waste bottles
1 ml. pipet
12 150 ml. beakers
pan
forceps
48 100 ml. beakers
clothespin

Materials

distilled water
2.5% NaH_2PO_4 solution
5.05% KNO_3 solution
2.5% K_2SO_4 solution
2.5% KH_2PO_4 solution
5.0% $NaNO_3$ solution
10.0% cane sugar solution
10^{-3} (3N) HC1 (pH = 3) solution
X-ray film fixative
1.25% Na_2SO_4 solution
1.25% $MgSO_4$ solution
0.01% NaCl solution
0.001% $FeCl_3$ solution
1.25% $MgCl_2$ solution
5.0% KCl solution
0.025 mg/ml solution Mn
X-ray film developer
marking pen and pencil
70-90% alcohol
filter paper
aluminum foil
absorbent paper
pH test paper
plant press blotter paper
plastic wrap
X-ray film
black construction paper

10^{-4}M $CaSO_4$ solution which contains 2.5 mg. Fe/liter plus 10 μgFe^{59} per liter

Plants

Aspergillus niger
bean plants
barley roots

Special Preparations

1) Stock solutions of the minerals required for *Aspergillus niger* nutrition should be made available in stock bottles with 10 ml. pipets. Prepare by adding each of the following to a liter stock flask and then adding distilled water to make one liter of solution:

12.5 g. Na_2SO_4
12.5 g. $MgSO_4$
0.1 g. NaCl
0.01 g. $FeCl_3$
12.5 g. $MgCl_2$
50 g. KCl
25 g. NaH_2PO_4
50.5 g. KNO_3
25 g. K_2SO_4
25 g. KH_2PO_4
50 g. $NaNO_3$
100 g. cane sugar

2) To make the Fe^{59} stock solution, add 0.14 g. $CaSO_4$ to 1 liter of distilled water and then add 2.5 mg. Fe and 10 μg. Fe^{59}. Allow 500 ml. Fe^{59} stock solution per student group.

3) Fe^{59} is easily obtained in license exempt quantities of 5 ml. with low concentrations of acid or carrier. By law you can order no more than ten vials during any one time period. This radioactive source is suitable for most tracer studies, even though it is not recommended for very precise research use. The half life of Fe^{59} is only forty-five and one-half days; thus, you must specify the usage date when ordering. See appendix B for a supplier. Such suppliers also usually sell x-ray film and processing chemicals used in autoradiographic experiments.

4) X-ray film processing chemicals usually are sold as developing fluids A and B and fixative. There are many kits available which contain both the X-ray film and processing chemicals. Directions are printed on the package. If you wish to purchase materials separately, hydroquinone (trade name — Quinol) is a good developing agent, especially when teamed with Metol (Elon, Veritol, Genol, Pictol, Photol, Rhodol, Errol, Scalol, Satrapol, Mirol — it is sold under many trade names). Hydroquinone is 1-4$(OH)_2C_6H_4$, 1-4 dihydroxybenzene, and Metol is $HO{\cdot}C_6H_4{\cdot}NH{\cdot}CH3{\cdot}1/2H_2SO_4$. Common hypo, or sodium thiosulfate, is what is normally sold as a fixative. It is much easier to purchase these as kits and to follow package directions.

5) Most schools have a darkroom set up for journalism and yearbook photographic activities. It usually is easy to arrange for time in the darkroom for science use. A darkroom usually will have many pieces of equipment and materials required for autoradiography, in which case you will not have to worry about a clothesline, clothespins, a water source, developing pans, etc.

6) X-ray film is widely available from many sources, including biological supply houses.

7) School art or industrial arts rooms may have a furnace with a 550° C. capability. If this is not available at the high school, search your locality for colleges with such a furnace, or amateur potters with kilns.

8) If your school does not have a scintillation well counter, one probably will be available at local institutions of higher learning or research oriented private companies.

9) Plant presses are available from most biological supply houses, or you can make your own.

10) *Aspergillus niger*: grow one slant of this bread mold for every two laboratory groups. Inoculate nutrient agar slants with mold one week before the start of the experiment. Incubate. See laboratories fourteen and seventeen for directions on preparing slants and on identifying *Aspergillus niger*.

11) Barley roots: germinate barley seeds on a mesh screen over a container filled with tap water. Run water into the container very slowly to maintain freshness. The roots will grow through the screen into the water so that they can be easily snipped off below the screen at the beginning of the experiment. Start germinating the barley seeds ten days before start of the experiment. By using only tap water for germination, the roots will have a low salt content, which promotes the conditions for good iron uptake.

If you were to germinate the barley roots on a screen over a complete nutrient solution (see optional activity in this laboratory for information on complete nutrient requirements), you would have produced high salt barley roots.

12) Bean plants must be started five weeks before the start of the experiment on mineral translocation. Germinate bean seeds on filter paper soaked in a complete nutrient solution (see the optional activity in this laboratory).

When the beans have germinated, grow them without soil for four more weeks. See the optional activity in this laboratory for the nutrients required for complete soilless growth. You may purchase the complete solution in most biological supply catalogues, or you may make your own following the directions given in this laboratory. If you make your own solution, omit the iron containing chemical and add Fe^{59} instead. Either way, add 1 ml. Fe^{59} per liter of nutrient solution. Grow two bean seedlings for each student group.

After the bean seedlings have grown for two weeks in the complete nutrient solution containing Fe^{59}, remove half the bean seedlings from the solution and grow them in complete nutrient solution without Fe^{59}. In this fashion you will have one bean seedling for each student group grown for two weeks in a radioactive solution, and one bean seedling grown for four weeks in a radioactive solution.

Time Required:

The mineral absorption, use, and translocation in plants laboratory requires seven full classroom hours, as well as one partial hour, in addition to preparations, discussion, and review.

Procedure: Hour 1

Students need the following: distilled water, solutions of 1.25% Na_2SO_4, 2.5% NaH_2SO_4, 1.25% $MgSO_4$, 5.05% KNO_3, 0.01% NaCl, 2.5% K_2SO_4, 0.001% $FeCl_3$, 2.5% KH_2PO_4, 1.25% $MgCl_2$, 5.0% $NaNO_3$, 5.0% KCl, and 10.0% cane sugar, 16 sterlizable storage jars with caps, and marking pen and pencil.

During this laboratory hour you will prepare eight mineral nutrient solutions. One solution will be complete, and the other seven will lack one mineral required for the culture of black bread mold, *Aspergillus niger*.

Steps

A. Label sixteen culture jars, two for each type of nutrient medium shown below in figure one, as well as with your name and today's date. Make up the nutrient solutions from stock solutions available from your instructor. Pipet 10 ml. of each stock solution, shown in figure one, into the appropriate culture jar. You will have sixteen culture jars labeled and filled, with eight pairs each containing 50 ml. of different nutrient solutions.

Complete	**Minus K**	**Minus N**	**Minus P**	**Minus Mg**	**Minus Fe**	**Minus S**	**Minus C**
KNO_3	$NaNO_3$	KCl	KNO_3	KNO_3	KNO_3	KNO_3	KNO_3
KH_2PO_4	NaH_2PO_4	KH_2PO_4	K_2SO_4	KH_2PO_4	KH_2PO_4	KH_2PO_4	KH_2PO_4
$MgSO_4$	$MgSO_4$	$MgSO_4$	$MgSO_4$	Na_2SO_4	$MgSO_4$	$MgCl_2$	$MgSO_4$
$FeCl_3$	$FeCl_3$	$FeCl_3$	$FeCl_3$	$FeCl_3$	$NaCl$	$FeCl_3$	$FeCl_3$
Sugar	Sugar	Sugar	Sugar	Sugar	Sugar	Sugar	Distilled Water

Figure 1

B. Cap your sixteen culture jars, but loosely so they do not seal while cooling. Sterlize them either with an autoclave, or a pressure cooker on a stove. Sterilize at 15 pounds pressure for 15 minutes. Leave the jars to cool to room temperature for at least twenty-four hours.

Procedure: Partial Hour 2

Students need the following: set up from hour one, cultures of *Aspergillus niger*, inoculating loop, flame source, distilled water, 1 ml. pipet, 10 ml. graduated cylinder, and pH test paper.

During this laboratory hour you will inoculate your mineral solutions with *Aspergillus niger*.

Steps

A. Read the pH of all sixteen culture jars. Do this with previously unopened tubes of pH paper, and without undue handling, to minimize the possibility of contamination.

1. Record the pH of each solution in the chart, figure two.

Solution	**pH of Jar #1**	**pH of Jar #2**
Complete		
Minus K		
Minus N		
Minus P		
Minus Mg		
Minus Fe		
Minus S		
Minus C		

Figure 2

B. Sterilize an inoculating loop in an open flame, and allow it to cool. Remove as many spores as you can with the inoculating loop from a slant of *Aspergillus niger*. The loop should be full of spores. Make a suspension of these spores in 10 ml. sterile distilled water (the suspension will be dark if enough spores have been introduced). Add 0.1 ml. of this suspension (about 2 drops) to each culture jar. Place the culture jars, loosely capped, in a warm cabinet or incubator for five to seven days.

Procedure: Hour 3

Students need the following: barley roots, distilled water, scissors, filter paper, scale or balance, 360 ml. Fe^{59} stock solution, HCl, waste container for radioactive solid materials, 4 radioactive waste bottles labeled #1 through #4, 9 150-ml. beakers, 9 evaporating dishes, pencil, glass stirring rod, incubator, and oven.

During this laboratory hour you will start tests on the absorbence of iron by barley roots. You will time the course of absorption, and determine how temperature affects absorption rate.

Steps

A. **This experiment is likely to be your first experience with radioactive substances. Before beginning work with any radioactive material, know and employ such precautions as the following:**

1). Label all containers holding isotopes with the warning tape or labels provided with their purchase. If not provided by the supplier, make an obvious label.

2). Monitor the area with a survey meter during and after the experiment (the scintillation well counter required for this laboratory can perform this function).

3). Discard paper or other solid waste materials into a properly labeled waste can for radioactive solid materials. Transfer liquid waste to properly labeled radioactive liquid waste bottles.

4). Avoid pipetting by mouth. Avoid transfer of radioactive substances to the mouth by careless hand movements to the lips, or any other way.

5). Splashes or spills on skin or clothing are to be immediately cleansed with rigorous washing and copious use of water.

6). Handle solutions over pans lined with absorbent paper. Clean up any spills at once with absorbent paper and discard paper into labeled waste can.

7). Arrange with custodial staff to discard radioactive solid and liquid wastes according to local regulations and laws.

B. Snip off barley roots which have been growing through a wire screen. Wash them thoroughly in distilled water, mix them well, and blot excess moisture off with filter paper. Weigh out nine 2.0 g. portions of barley roots.

C. **Time Course of Absorption:** fill nine 150 ml. beakers with 40 ml. Fe^{59} stock solution and 10 ml. distilled water each. Place one 150 ml. beaker in the refrigerator, leave one at room temperature, and place one in an incubator set at 27° C. Label the three beakers with the temperature they are placed in, as well as with your name and today's date. Label the other six beakers with the absorbence time you will test, namely: 1 minute, 5 minutes, 10 minutes, 20 minutes, 30 minutes, and 24 hours. Also label with your name and today's date. You are now ready to run an experiment testing the time course of iron absorption in barley roots. At zero hour, place 2.0 g. portions of barley roots in the six beakers labeled with times.

D. At exactly 1 minute, decant the Fe^{59} solution into waste bottle #1, using a glass stirring rod to hold the roots in the beaker. Rinse the roots three successive times in HCl, using about 25 ml. HCl each time. Decant the used HCl in waste bottles #2, #3, and #4, respectively.

E. Blot the drained roots and transfer them to a small evaporating dish. Label the evaporating dish as to content, your name, and today's date. Use a pencil to mark the dish because graphite will not disappear when subjected to the high temperatures of a furnace. Dry the roots in an oven about 80° to 90° C. for the remainder of the laboratory hour, or until roots are thoroughly dry.

F. Repeat steps D and E with the beaker labeled 5 minutes at 5 minutes after zero hour.

G. Repeat steps D and E with the beaker labeled 10 minutes at 10 minutes after zero hour.

H. Repeat steps D and E with the beaker labeled 20 minutes at 20 minutes after zero hour.

I. Repeat steps D and E with the beaker labeled 30 minutes at 30 minutes after zero hour.

J. Leave the beaker marked 24 hours overnight at room temperature.

K. **The Effects of Temperature on Absorption:** place the remaining three portions of barley roots in the beaker to be left at room temperature, the beaker to be placed in the refrigerator, and the beaker to be placed in the incubator. Leave them at those temperatures for 24 hours.

Procedure: Hour 4

Students need the following: entire set up from hour three, distilled water, filter paper, Fe^{59} stock solution, manganese solution, 3 150 ml. beakers, and a drying oven.

During this laboratory hour you will continue the test on the absorbence of iron by barley roots. You will complete timing the course of absorption, and determine how temperature affects absorption rate. You will also set up an experiment to test the effect of a competing ion on iron absorption.

Steps

A. Repeat steps D and E of laboratory hour three with the beaker labeled 24 hours.

B. Repeat steps D and E of laboratory hour three with the three beakers at different temperatures, namely: refrigerator, incubator, and room temperature.

C. **The Effects of a Competing Ion on Absorption:** fill and label three 150 ml. beakers with the solutions listed in figure three, as well as with your name and today's date.

Solution	Beaker #1	Beaker #2	Beaker #3
Fe^{59} Stock Solution	40 ml.	40 ml.	40 ml.
Distilled Water	9.8 ml.	9.8 ml.	9.8 ml.
Manganese Solution (0.025 mg/ml)	0.2 ml.	1.0 ml.	10.0 ml.

Figure 3

D. Follow step B of hour three of this laboratory and make three more 2.0 gram portions of snipped barley roots. Place 2.0 gram portions of snipped barley roots in each of the three beakers filled with the above solutions, and leave the experiment at room temperature for twenty-four hours.

Procedure: Hour 5

Students need the following: entire set ups from hours three and four, furnace at 550° C., 1 bean plant grown 4 weeks with Fe^{59}, 1 bean plant grown 2 weeks with Fe^{59} and 2 weeks without, plant press with blotters, darkroom, plastic wrap, aluminum foil, x-ray film, black paper, and refrigerator.

During this laboratory hour you will ash the results of your experiments on iron absorption in barley roots, and set up an experiment to record the translocation of iron in bean plants.

Steps

A. Repeat steps D and E of laboratory hour three with the solution beakers labeled #1 through #3 prepared during hour four.

B. Take all twelve labeled evaporating dishes filled with dried barley root residue and ash them to grey-white in a 550° C. furnace for twenty to thirty minutes. Allow the ash to cool for twenty-four hours.

C. **Mineral Translocation:** put on the same plant press sheet a plant grown for 4 weeks in a nutrient solution with Fe^{59}, and a plant grown for 2 weeks in the same solution and then for 2 weeks in a normal complete nutrient solution. Label appropriately. Place a plant press blotter top and bottom and cover the package with plastic wrap. Take the package to a darkroom. Next, in complete darkness, place a sheet of x-ray film behind one of the blotters. Wrap the whole package, plants, sheet, blotters, plastic wrap, and x-ray film, in aluminum foil and black paper and insert the whole package in the plant press. Store in a cool, dark place for two or three days.

Procedure: Hour 6

Students need the following: twelve evaporating dishes with barley root ash, 3N HCl, warming oven, 13 15x140 mm. counting tubes with screw caps, pipet, pans, paper toweling, and a scintillating well counter.

During this laboratory hour you will determine the radioactivity of the treated barley roots. This must be accomplished entirely in one hour, or else all counts must be corrected to compensate for further radioactive decay. Remember, the half life of Fe^{59} is forty-five and a half days.

Steps

A. Using three successive 1 ml. portions of warm (about 50° - 60° C.) 3N HCl to dissolve the ash, transfer by pipetting the ash in each of the twelve evaporating dishes, or the soluble part thereof, to flat-bottomed 15x140 mm. counting tubes with screw caps. Each counting tube ultimately should contain 3.0 ml. ash solution. Do all transfer over pans layered with absorbent paper. Label each tube with original data.

B. Fill the remaining counting tube with 3.0 ml. 3N HCl. This thirteenth tube will be used to obtain a background count which will be subtracted from the other twelve readings, to obtain a figure for any radioactive increase in the barley roots.

C. Using the scintillation well counter, obtain a radioactive count from all tubes. (Most scintillation counters have simple directions printed directly on the machine).

All radiation detectors that respond by emitting a flash of visible light are classified as scintillators or fluors. Most scintillation counters today combine a fluorescent tube and a multiplier phototube. The total light output of the fluorescent tube is functionally related to the total energy lost by a moving charged particle in the tube. If the fluorescent tube makes good optical contact with the multiplier phototube, the light produced in the fluorescent tube liberates electrons photoelectronically from the photosensitive surface on the face of the tube. By applying appropriate voltage increments between successive dynodes of the tube, these electrons are accelerated, and at each dynode secondary electrons are produced. Because of this electron miltiplication, the final output of the tube is an electrical pulse of sufficient magnitude to be counted. The light is counted electronically and read as cpm, or counts per minute.

2. Record the cpm for each of the twelve samples in the chart, figure four. The background count will be the count from the thirteenth tube, filled only with 3N HCl, which will be subtracted from each sample to obtain a net cpm for each sample of ashed barley root.

Sample	**CPM**	**3N HCl Count**	**Net CPM**
1 Minute			
5 Minutes			
10 Minutes			
20 Minutes			
30 Minutes			
24 Hours			
Temperature			
Refrigerator ____° C.			
Room ____° C.			
Incubator ____° C.			
Competing Ion			
0.01% Manganese			
0.05% Manganese			
0.50% Manganese			

Figure 4

3. Graph these results in figure five. Three simple graphs will be necessary, with one axis of all three being cpm's, and the other axis being time, temperature, or manganese concentration, respectively.

Figure 5

4. Did the length of time that the barley roots were exposed to the Fe^{59} correlate uniformly with Fe^{59} uptake? Why or why not?

5. How did temperature affect the uptake of Fe^{59}?

6. How did the presence of a competing ion affect the uptake of Fe^{59} by barley roots? Compare the room temperature or timed twenty-four hour absorption rate with the absorption rates in the presence of 0.01% Mn, 0.05% Mn, and 0.50% Mn.

7. From the data you have collected, what conditions favor optimum iron absorption by a plant?

Procedure: Hour 7

Students need the following: 16 culture jars containing *Aspergillus niger* from hour one, forceps, 70 - 90% alcohol, distilled water, 16 watch glasses, oven at 72° C., 48 100 ml. beakers, balance or scale, paper toweling or blotting paper, filter paper, pH paper, and a marking pen.

During this laboratory hour you will clean and dry the *Aspergillus niger* grown under varying nutrient conditions in culture jars.

Steps

A. Observe the sixteen culture dishes with *Aspergillus niger*.

8. Make notes on the external appearance of each batch in the spaces provided on the following page.

Solution

Complete #1 & #2 ______________________________

Minus K #3 & #4 ______________________________

Minus N #5 & #6 ______________________________

Minus P #7 & #8 ______________________________

Minus Mg #9 & #10 ______________________________

Minus Fe #11 & #12 ______________________________

Minus S #13 & #14 ______________________________

Minus C #15 & #16 ______________________________

9. Record the pH of each solution in the chart, figure six.

Solution	pH of Jar #1	pH of Jar #2
Complete		
Minus K		
Minus N		
Minus P		
Minus Mg		
Minus Fe		
Minus S		
Minus C		

Figure 6

10. How do these results compare with those recorded in figure two? Explain any differences.

B. Label forty-eight 100 ml. beakers in a manner similar to each of the sixteen culture dishes, namely: six beakers complete, six beakers minus K, six beakers minus N, etc. Additionally label sixteen of the beakers with the word "alcohol" (taking two from each set of nutrient solution type), and fill each of the sixteen with about fifty ml. 70 - 90% alcohol. Label sixteen of them with the words "tap water" (again taking two from each nutrient solution type), and fill each of those sixteen with about fifty ml. tap water. Label the remaining sixteen with the words "distilled water" and fill each of those with about 50 ml. distilled water each.

C. Pick out the mycelium from each culture dish with forceps, and place each mat in a beaker with alcohol for thirty seconds. Drain off the alcohol, and transfer the mats to beakers which contain tap water.

D. The mycelial mats should remain in the tap water for two minutes, during which time you should agitate the beaker and flow additional tap water in and out of the beaker. At the end of two minutes, drain off the tap water, and transfer the mats to beakers which contain distilled water.

F. Number watchglasses one through sixteen. Weigh each watchglass and record the weight on it. Place the drained and blotted mats on the appropriate watchglasses. Dry the mats on watchglasses in a 72° C. oven for about twenty-four hours.

Procedure: Hour 8

Students need the following: dried mycelial mats on watchglasses from hour seven, dessicator, balance or scale, Fe^{59} containing plants in plant press from hour six, darkroom, developer and fixer, and clothesline and clothespins.

During this laboratory hour you will cool and weigh the *Aspergillus niger* mycelial mats, and develop an autoradiograph of your Fe^{59} containing bean seedlings.

Steps

A. Cool the sixteen oven dried mycelial mats in a dessicator, and weigh each of them.

11. Record the weights of each mat in the chart, figure seven.

Solution	#1 Weight	#2 Weight
Complete		
Minus K		
Minus N		
Minus P		
Minus Mg		
Minus Fe		
Minus S		
Minus C		

Figure 7

12. Which nutrient bath grew the most *Aspergillus niger?* ____________________

13. Which nutrient bath grew the least *Aspergillus niger?* ____________________

14. Which minerals seem to be the most vital to the growth of *Aspergillus niger?* Why?

____

____

____

14. Which minerals seem to be the most vital to the growth of *Aspergillus niger?* Why?

B. In the dark, remove the x-ray film from the plant press and bean seedlings. Develop the film using the developing fluid and fixative supplied by your instructor. Wash the film 30 minutes in tap water, and then hang it up to dry. Save the plant material to compare with the autoradiograph.

15. Compare and explain the results with the two plants.

Optional Activity

Students may wish to experiment with soilless growth on vascular plants. The following are directions on how to set up an experiment on mineral deficiency cultures with a vascular plant. The plant chosen for this experiment is a tomato plant, but corn plants may be substituted, as their mineral requirements are similar. These solutions are based on Hoagland's Solution #1, which was first published in a California Agricultural Experimental Station Circular, #347, in 1938.

Hoagland's complete and mineral deficiency solutions may also be purchased from biological supply houses.

A. Wash 18 one quart mason jars in lukewarm water, rinse three or more times with tap water, plus three times with distilled water. The jars are usually painted or whitewashed to prevent sunlight striking the roots. Prepare solutions in duplicate in these jars, using the compositions as given in the table, figure eight, proceeding as follows. Fill the jar three quarters full of distilled water, then add the mineral components one by one with graduated pipettes, stirring with a large stirring rod after each addition. Bring the level in each jar to within three centimeters of the rim with the addition of distilled water, and stir again. Fit a cork to each jar. Label the jars as to type of solution, today's date, and your name.

Milliliters of Stock Solutions per One Quart Jar

Stock Solution	Complete	-N	-P	-K	-Ca	-Mg	-S	-Fe	-Micro-nutrient
KH_2PO_4	1	1	-	-	1	1	1	1	1
KNO_3	5	-	5	-	5	5	5	5	5
$Ca(NO_3)_2$	5	-	5	5	-	5	5	5	5
$MgSO_4$	2	2	2	2	2	-	-	2	2
KCl	-	5	1	-	-	-	-	-	-
$CaC1_2$	-	5	-	-	-	-	-	-	-
NaH_2PO_4	-	-	-	1	-	-	-	-	-
$NaNO_3$	-	-	-	5	10	-	-	-	-
Na_2SO_4	-	-	-	-	-	2	-	-	-
$MgCl_2$	-	-	-	-	-	-	2	-	-
FeEDTA	1	1	1	1	1	1	1	-	1
Micro-nutrients	1	1	1	1	1	1	1	1	-

Figure 8

The iron source, FeEDTA, is the iron chelate of ethylene-di-amine-tetra-acetic acid, made up to contain 5 mg. Fe per ml. The micro-nutrients are compiled using Haas and Reed's "A to Z" solution which is made by weighing out the following ingredients and dissolving them in 1 liter of distilled water:

H_3BO_3	0.6 g.	KBr	0.03 g.
$MnCl_2 4H_2O$	0.4g.	$Co(NO_3)_2 6H_2O$	0.05 g.
$ZnSO_4$	0.05g.	LiCl	0.03 g.
$CuSO_4 5H_2O$	0.05 g.	TiO_2	0.03 g.
$Al_2(SO_4)_3$	0.05 g.	$SnCl_2 2H_2O$	0.03 g.
KI	0.03 g.	$NiSO_4 6H_2O$	0.05 g.

B. Select 18 tomato seedlings for uniformity of shoot and root length. Record the average lengths. Also select them for uniform sturdiness and vigor. Drill holes in the corks of the jars so that the seedlings may be inserted in the holes. Also drill a small hole for the aerator. Insert the seedlings root first into the corks, secure them firmly but not too tightly with small wads of non-absorbent cotton, and place them in the jars. Avoid letting the roots dry out during these manipulations.

C. Place the jars on a designated area of a greenhouse bench, insert capillary glass aerators, connect the aerators to the air-line, and turn on a fine stream of bubbles (aquaria air pumps will suffice). Leave the plants for two or three weeks.

D. Nutrient solutions should be changed every two to three weeks, and the experiment should be continued until deficiency symptoms are obvious. Normally this will take six to eight weeks.

E. At harvest, cut the tomato shoots off just above the corks. Place root and shoot in separate labeled paper bags, dry for 48 hours in a 70° C. ventilated oven, and weigh. Report the weights, average the two plants per solution, and compare this average against the average weight of the plants grown in a complete nutrient solution.

Students may also wish to record the pH of new and used nutrient solutions. Tomato plants grow best in a slightly acidic environment, such as pH 5 to 5.5. They have difficulty growing in a pH of 7.0 or greater.

Resources

Biddulph, S. and O. Biddulph. "The Circulatory System of Plants." *Scientific American*, February 1959.

Epstein, E. "Roots." *Scientific American*, May 1973.

Niklas, Karl J. "Computer-simulated Plant Evolution." *Scientific American*, March 1986.

Rick, Charles M. "The Tomato." *Scientific American*, August 1978.

Terminology

Students should understand the following terms and concepts prior to taking the unit review:

autoradiography

translocation

Review

30. Mineral Absorption, Use, and Translocation in Plants

Name ______________________________

Date ______________________________

Essay

1. What is meant by translocation? Why does it occur?

2. Name the macro- and micronutrients of plants. What are their respective roles in plant growth and function?

3. What are the advantages of using radioactive tracers in plant physiology studies?

Answers Found: essay 1: p 454 - 455; essay 2: p 453 - 454; essay 3: p 454 & 455; as well as the laboratory proper.

Appendix A:

Glossary

Abiotic: not living.

Absorbance: in spectrophotometry, the degree to which light is absorbed. Absorbance = 2 − log × % transmittance, where transmittance is the measure of light which emerges from an object or solution in relation to the amount of light which enters it.

Acid Fast Stain: used in microbiology to determine those bacteria whose cell walls retain stains even when exposed to acid-alcohol solutions.

Action: in anatomy, a muscle's function. The action of a muscle is to move a bone in a particular way.

Action Spectrum: the range of wavelengths which elicit a response in organisms and the intensity thereof.

Activator: something which initiates a reaction.

Active Site: the region of an enzyme surface that binds the substrate during reactions catalyzed by the enzyme.

Aecia: among rusts, a cup at [illegible] surface of the host, in which dicaryotic spores called aeciospores are borne.

Aerobe: an organism which lives only in the presence of oxygen.

Agar: a gelatinous, colloidal extract of the red alga genera *Gelidium*, *Gracilaria*, or *Eucheuma* which is used as a gelling and stabilizing agent in culture media and foods.

Agaricus campestris: the common field mushroom.

Agarose: a polysaccharide obtained from agar which is used as a supporting medium in chromatography and gel electrophoresis.

Agonistic: being aggressive or hostile in behavior.

AIDS: acquired immune deficiency syndrome. Fatal disease caused by a retrovirus.

Akinete: an algal spore produced by transformation of a whole vegetative cell, with the original cell wall forming part or all of the spore wall.

Albumen: egg white.

Allantois: the waste sac of an embryo. In later embryonic development it combines with the chorion to form the chorioallantoic membrane which additionally functions in embryonic respiration.

Allergy: an exaggerated or pathological immune response to substances which normally do not affect most people.

Alternation of Generations: the occurrence of two or more morphological forms differently produced in the life cycle of an organism. This usually involves the regular alternation of a sexual with an asexual generation, but it also may involve the alternation of diploid and haploid generations.

Amnion: fluid which cushions and protects embryos. It is found in a sac which surrounds the embryo called the amniotic sac.

Ampholyte: a compound which may react either as a base or as an acid.

Amphoteric Protein: a protein capable of reacting either as a base or as an acid.

Anaerobe: an organism which lives in the absence of oxygen.

Angstrom: a unit of wavelength measurement used especially for light and symbolized as Å. A unit of length equal to one ten-billionth of a meter.

Animalia: in the classification of living organisms, the animal kingdom.

Antheridium: a specialized cell or multicellular structure, within which one or more sperms are produced.

Antibiotic: against life. A substance produced by microorganisms able to inhibit or kill bacteria by destroying cell walls.

Antibody: protein immunoglobulins produced by *B* lymphocytes in response to a specific antigen.

Antigen: a protein or carbohydrate which stimulates an immune response.

Aponeuroses: thin, flattened cords or tendons of deep fascia.

Appendicular: in the skeletal system, of or relating to the appendages.

Archegonium: a specialized structure within which an egg is produced.

Ascomycetaceae: also known as the cup fungi. The class of higher fungi, such as yeasts and molds, with septate hyphae and spores formed in asci, or cups.

Ascus: a membranous oval or tubular spore case of an ascomycete.

Aseptic Technique: a procedure which is free from contamination by living organisms.

Asexual: without sex. Any reproductive process, such as budding or fission, which does not involve the union of gametes.

ATP: adenosine tri-phosphate. The principal energy carrying compound of cells.

Autoimmune Disease: results from antibodies attacking molecules, cells, or tissues of the very organism which produces the antibodies.

Autoradiography: a technique whereby the location of an introduced radioactive element is detected within an organism or system by radiation which exposes (blackens) photograhic film placed next to it.

Auxin: a group of plant hormones which have a variety of growth regulating effects, including the promotion of cell elongation.

Axial: in the skeletal system, of or relating to the trunk, the main axis, of the body.

Azolla: a common water fern of the order *Marsileales*, known for its symbiotic relationship with the blue-green algae *Anabaena*, which allows it to fix atmospheric nitrogen.

Balmer Series: all lines in the spectrum of hydrogen which lie in the visible and near ultraviolet regions, or between 2,500 Å and 7,000 Å.

Base Line: a line used in chromatography to place samples and to measure movements within the system.

Basidiomycetaceae: also known as the club fungi. The class of higher fungi, such as rusts, smuts, mushrooms and puffballs, having septate hyphae and bearing spores on a basidium.

Basidium: the structure on a basidiomycete in which nuclear fusion occurs followed by meiosis, and on which, typically, four basidiospores are borne. These are sexual spores born externally.

Bed: in electrophoresis, the body or frame of the machine on which electrophoresis occurs.

Beebread: a mixture of pollen and honey made to feed worker bee larvae.

Beer's Law: in spectrophotometry, absorbance equals the reciprocal of transmittance.

Berberis: the genus of barberry plants. A common vector for rusts.

Biogenesis: the development of life from pre-existing life.

Bioluminescence: the emission of light by living organisms.

Biotic: living.

Blank: in microbiology, a sterilized test tube partially filled with water.

Blastodisc: a flattened blastoderm, or cells in the blastula stage of embryonic development, caused by discoidal cleavage. This type of blastula is typical of fishes, birds, reptiles, and monotremes.

Blastopore: the "lip" of the embryonic gastrula stage, which is the opening to the archenteron, or primitive cavity of the gastrula, formed when the hollow ball of cells of the blastula stage caves inward during development. The blastopore is believed to be the site of many organizer cells which determine growth and differentiation in the embryo.

Blastula: an embryonic stage of development in which cells are arranged in a hollow ball-like structure around a central cavity, or blastocoel.

Brownian Movement: not organized movement. A random movement of microscopic particles suspended in liquids or gases resulting from the impact of fluid molecules surrounding the particles.

Bryophyta: any of a phylum of nonflowering plants comprising the mosses and liverworts.

Budding: unequal cell division, as in yeasts.

Calcite: a mineral, $CaCo_3$, consisting of calcium carbonate, crystallized in hexagonal form.

Calyptra: in bryophytes, the structure derived partly or totally from the archegonium, which caps or immediately surrounds the sporophyte.

Cambium: a lateral meristem, a ring of cells which, from successive mitoses, produces xylem internally and phloem externally.

Cancer: a group of diseases characterized by unrestrained cell growth which spreads locally by invasion and systematically by metastasis.

Capsule: the sporangium of a bryophyte.

Catalyst: a substance, such as an enzyme, which initiates and allows a reaction to proceed under conditions different than otherwise possible. It participates in the reaction but is not used up by the reaction.

Cell: an organized unit of protoplasm, bounded by a membrane or wall.

Cell Line: continuing growth of one cell type in a cell tissue culture.

Cell Tissue Culture: growing cells *in vitro* outside the organism of origin.

Chalaza: twisted strands of albumen.

Chlorophyta: the phylum of green algae in the algae division of the sub-kingdom *Thallophyta* in the plant kingdom.

Chorion: the outer membrane of the amniotic sac.

Chromatography: a process in which a chemical mixture, carried by a liquid or gas, is separated into components as a result of differential distribution of the solutes, as they flow around or over a stationary liquid or solid.

Chrysophyta: the phylum of yellow-green or golden brown algae in the algae division of the sub-kingdom *Thalophyta* in the plant kingdom.

Chytridiales: the order of unicellular algal-like fungi of the phycomycete class of the phylum *Eumycophyta* in the fungi division of the sub-kingdom *Thallophyta* in the plant kingdom.

Ciliata: a phylum of protozoans which move by means of cilia.

Claviceps purpuerea: ergot of rye, wheat, and other cereals. An ascomycete in the fungi division of the sub-kingdom *Thallophyta* in the plant kingdom.

Cleavage: synchronized, mitotic embryonic cell division, in which cells stick together after division, thus forming multicellular organisms.

Coacervate: an aggregate of colloidal droplets or polymers held together by electrostatic forces.

Cocci: spherical bacteria.

Coelom: the cavity between the digestive tract and the body wall in all animals above the lower worms.

Coenocytic: an organism which has the nuclei scattered in a continuous, usually filamentous, protoplast that is not divided into definite cells.

Colony: a group of organisms living together in close association.

Columella: the axis of the capsule in mosses and some liverworts. Also, the central sterile portion of the sporangium in some fungi.

Column Chromatography: the technique whereby chromatographic separation is accomplished by filling a glass tube with material such as plastic resin beads, which are electrically charged, and pouring the material to be analyzed down the column in solution. The charge on the beads separates the material differentially, thereby fractionating the material. The material is then recovered by analyzing the material in its order of movement down the column.

Complement Protein: an enzymatic protein which destroys foreign invaders marked by *B* lymphocytes.

Compound Microscope: a microscope consisting of an objective and an eyepiece mounted in a drawtube.

Confluency: in cell tissue culture, the growing together of cells.

Conidium: an asexual spore produced on a conidiophore.

Conifer: any of a phylum *(Coniferophyta)* of mostly evergreen trees and shrubs including forms with true cones (as pines) and others (as yews) with fruit.

Conjugation: a one way transfer of DNA between bacteria in cellular contact. Also, temporary cytoplasmic union with an exchange of nuclear material among ciliated protozoans. Also, the fusion of iosgametes.

Coplin Jar: jars with lids used to hold glass slides during staining procedures or to hold glass plates during chromatography procedures.

Cork: a waterproofing protective tissue, dead when functional, characterized by the presence of a complex fatty substance called suberin.

Cortex: in plants, the tissue between the central cylinder and the epidermis of a stem or root.

Counter-stain: a second stain used to stain parts of an organism which did not stain during the first staining.

Critical Distance: the narrow band of personal space in which an intruder forces an animal to fight or flee.

Critical Light Length: the amount of light, or lack thereof, which stimulates the flowering process in many plants.

Cuvette: a small, transparent, laboratory tube. Cuvettes used in spectrophotometry are rectangular.

Cyanophyta: the phylum of prokaryotic, blue-green algae in the algae division of the sub-kingdom *Thalophyta* in the plant kingdom.

Cycad: any of a phylum of gymnosperms *(Cycadophyta)* resembling palms but reproducing by means of spermatozoids.

Dark Reaction: the phase of photosynthesis that does not require light. It involves the reduction of carbon dioxide to form carbohydrates.

Dead Air Space: in an egg, the air space between the two shell membranes.

Decant: to pour or draw off without disturbing the lower layers or sediment.

Decolorizer: in microbiological staining procedures, the compound, usually alcohol or acid alcohol, that removes excess stain from the organism.

Deep: in microbiology, a test tube partially filled with plain or nutrient agar.

Denaturation: to deprive something of its natural qualities. To modify the molecular structure of a compund or organism which is biologically active in order to change that specific activity. This may be accomplished by heat, acid, alkali, or ultraviolet radiation.

Dextrorotatory: to turn clockwise, or toward the right.

Diasteriomer: a stereoisomer that does not have a mirror image.

Differential Staining: in microbiology, the use of two or more stains to show a difference in the constituents of a cell.

Diploid: 2N, or having two sets of chromosomes for every trait or characteristic.

Dye: a stain or coloring matter which may be soluble or insoluble.

Egg: the 1N, or haploid, reproductive cell consisting of a ovum, nutritive material, and a covering.

Egg Canal: a bird's oviduct.

Electromagnetic Radiation: a series of electromagnetic waves, or vibrating electrical charges in a magnetic field. These waves exist in lengths from gamma rays to radio waves.

Electron Microscope: a microscope in which a beam of electrons, focused by means of an electron lens, is used to produce an enlarged image of a tiny object on a fluorescent screen or photographic plate.

Electrophoretic Mobility: utilized in electrophoretic separation by subjecting substances to an electric current, which causes them to differentially move on the substrate.

Electrophoresis: the movement of suspended particles through a fluid or gel under the action of an electromotive force applied to electrodes in contact with the suspension.

Elution: the removal of an adsorbed material by means of a solvent.

Embryology: the study of young animals or plant sporophytes while still contained within a protective structure such as an egg, uterus, or seed.

Enantiomer: either of a pair of chemical compounds or crystals whose molecular structures are the mirror image of one another.

Enzyme: a complex protein produced by a living cell which catalyzes specific biochemical reactions. An organic catalyst.

Epithelial Cells: cells derived from membranous tissue which form junctions with each other when grown in cell tissue cultures.

Epitope: a center, or specific molecular pattern, for antibody attachment to a virus or bacterium.

Erg: a cgs (centimeter/gram/second) unit of work equal to the work done by a force of one dyne acting through a distance of one centimeter.

Ethology: the study of animal behavior in nature.

Eukaryotic: "true" cells. An organism composed of cell(s) which have distinct nuclei and membranous organelles.

Eumycophyta: "true" fungi, of the fungi division of the *Thallophyta*. Fungi with definite walls which are usually chitinous or sometimes composed of cellulose.

Eusporangiate: plants in which the sporangium develops from a group of sporangial initial cells.

Excited State: the state of a physical system, such as an atom or a molecule, that is higher in energy than the ground state.

Extraembryonic: outside the embryo. Extraembryonic most often refers to those structures developed from the embryo which supply it with food, respiration, and waste removal.

Facultative Anaerobe: organisms capable of surviving either with or without oxygen.

Fascia: a sheet of connective tissue binding together body structures.

Ferment: a living organism that causes fermentation by enzymatic production. Also, to undergo fermentation.

Fermentation: an enzymatically controlled anaerobic breakdown or transformation of an energy rich compound. An example is the breakdown of a carbohydrate to carbon dioxide and alcohol.

Fertilization: fusion of a sperm with an egg.

Fibroblastic Cells: undifferentiated mesenchyme cells which give rise to connective tissue. These cells grow in single layers in cell tissue cultures.

Fiddlehead: an immature fern frond.

Filamentous: thread-like.

Filicales: fern order known as "true" ferns. All members are homosporus and leptosporangiate.

Flagellate: a unicellular or colonial organism, other than a bacterium, which moves by means of flagella (slender, motile, protoplasmic projections from the body of an organism).

Florigen: believed to be a plant hormone which regulates flowering.

Follicle: the egg and its yolk.

Foot: in botany, a base. In the *Psilophyta* and other lower vascular plants, a foot is the gametophyte base to which the sporophyte generation is attached.

Fractionation: separation into different portions.

Fragmentation: to break apart, or a separation into pieces.

Free Solution Electrophoresis: electrophoresis in which samples are fractionated in a U-tube filled with unstabilized buffer.

French Flag Theory: states that embryological differentiation is based upon a chemical gradient which operates through diffusion from a lead cell to others in a cell group based upon their position within the group.

Frond: a large leaf of many divisions, usually of a fern or palm tree.

Fungus: a parasitic or saprophytic lower plant that lacks chlorophyll. They include molds, rusts, mildews, smuts, mushrooms, puffballs, and yeasts.

Galvanometer: an instrument for detecting and measuring electric currents through movements of a magnetic needle, or of a coil in a magnetic field.

Gametangium: any structure in which gametes are born.

Gamete: any cell which is capable of fusing with another cell to form a new individual.

Gas Chromatography: chromatograhy in which the sample is moved in vapor form by a carrier gas, such as nitrogen or helium, through a column filled with liquid or a particulate solid. It is separated and stratified into its component parts by absorption in the column.

Gastromycetaceae: a class of higher fungi, such as puffballs and their allies, in the holobasidial group of the *Eumycophyta*. The hymenial layer is not exposed at maturity.

Gastrula: an early embryological stage consisting of two cell layers.

Gel: a colloid more solid than liquid.

Gel Electrophoresis: electrophoresis in which the subsrate is a gel, usually agarose or polyacrilimide.

Gemmae: an asexual reproductive body that becomes detached from the parent plant.

Genetic Variation: a deviation from the typical form and/or function in the biochemical code for heredity in organisms.

Gibberellin: one of a group of plant growth hormones, first discovered in a fungus, which promotes cellular elongation.

Gill: in mycology, one of the radiating plates on the underside of a mushroom cap. The gills support mushroom reproductive structures.

Glycolysis: the enzymatic breakdown of a carbohydrate through use of phosphate derivatives, with the production of pyruvic or lactic acid and storage of energy in such high energy phosphate bonds as ATP.

Gram Negative and Positive: in microbiology, gram positive bacteria hold a crystal violet, or purple, stain. Gram negative bacteria do not and must be counterstained, usually with safranin.

Gram Stain: a differential staining technique by which some bacteria remain colored and others are decolored and counterstained, depending on the structure of their cell walls.

Ground State: the energy level of a nucleus, atom, or molecule at the lowest possible level.

Guaiacol: a complex compound from the *Guaiacum*, tropical American trees and shrubs. It is used as a reducing agent.

Gymnosperm: any of a division of woody, vascular plants which reproduce by means of seed not enclosed in an ovary and that, in some instances, have motile spermatozoids.

Haploid: having half the genetic number of chromosomes as an organism's somatic cells.

Hemolysis: the bursting of red blood cells.

Heterogamete: having gametes of unequal size, such as sperm and egg.

Heterogamous: an organism which produces heterogametes.

Heterokaryon: a cell that contains two or more genetically dissimilar nuclei. This condition does occur naturally, such as in some algae and fungi, but it also refers to the first phase product of the laboratory creation of hybridomas.

Heterosporous: having spores of two sizes.

Hill Reaction: the light reaction of photosynthesis, in which electrons are transferred by chloroplasts to split water molecules and free oxygen.

Homosporous: having spores of one size.

Host: a living organism which provides food and/or lodging to a parasite.

Hybridoma: a hybrid cell produced by the fusion of an antibody-producing lymphocyte with a tumor cell and used to continuously culture a specific monoclonal antibody.

Hymenium: in botany, the spore bearing layer of a fungus consisting of a group of asci or basidia often interspersed with sterile structures.

Hypha: any single filament of a fungus.

Immune System: an organism's system of protecting self from invading bacteria, viruses, and other alien matter.

Immunoelectrophoresis: an electrophoretic technique whereby biological material is separated into fractions, and the fractions are then allowed to react with immune serum, following their diffusion through the electrophoretic bed.

Imprinting: a rapid learning process which takes place early in a social animal's life in which it learns to recognize its parents and own group.

In Vitro: growing cells which replicate outside the source organism.

Incident Beam (I_0): in spectrophotometry, light which has passed through the sample.

Indole Acetic Acid (IAA): one of a group of natural plant hormones called auxins which regulate and promote growth. Synthetic auxins, such as 2,4 D and 2,4,5 T, are long lasting and used extensively as herbicides.

Indusium: the covering on fern sori.

Infundibulum: the abdominal opening of an egg canal.

Insertion: the point where a muscle connects to a bone that it moves.

Integument: one of the one or two layers which partly encloses the megasporangial wall of an ovule. It is the forerunner of the seed coat.

Ion Exchange Chromatography: a chromatographic process in which ion exchange resins are used in column chromatography.

Ionization: causing a group of atoms or molecules to carry a positive or negative electric charge.

Isoelectric Focusing: in electrophoresis, the process of finding the isoelectric points in a compound.

Isoelectric Point: the point of zero difference in electric potential, or the pH at which an electrolyte, usually of a protein, will not migrate further in an electrical field.

Isogamete: a gamete which can fuse with another of similar size and appearance to form a zygote.

Isogamous: producing isogametes.

Isthmus: part of an egg canal where shell membranes are produced.

Kinetin: one of a group of plant hormones called cytokinens which promote cellular division.

Lactic Dehydrogenase (LDH) Isoenzyme: there are five isoenzymes of LDH, an enzyme that removes hydrogen ions from lactic acid. Isoenzymes are enzymes that catalyze the same reaction but migrate differently when undergoing electrophoresis. Their physical properties may or may not be different.

Lawn: in microbiology, bacterial growth on solid media which covers the entire surface area.

Leptosporangiate: plants in which the sporangium develops from a single sporangial initial cell.

Levorotatory: counterclockwise, or turning to the left.

Lichen: a complex of thallophytic plants composed of an alga and a fungus growing in symbiotic association on a solid surface, typically on rocks.

Life Cycle: the series of stages in form and function through which an organism passes during its lifetime.

Light Wavelength: a disturbance or variation which transfers light energy progressively from one point to the next. The part of the electromagneic radiation spectrum visible to humans.

Lipoprotein: a conjugated protein that is part protein and part lipid, or fat.

Liquid Media: in microbiology, a liquid substrate used to grow microorganisms.

Liverwort: a bryophyte, class *Hepaticae*, which resembles mosses but differs in reproduction, development, and in the structure of the gametophyte.

Luciferase: an enzyme that catalyzes the oxidation of luciferin.

Luciferin: a pigment in bioluminescent organisms which yields practically heatless light while undergoing oxidation.

Lycopsida: a plant phylum commonly known as club mosses or ground pines, in the division *Pteridophyta* of the sub-kingdom *Tracheophyta*.

Lymphocyte: colorless, weakly motile cells, produced in bone marrow, which are part of the immune defense system.

Magnum: part of an egg canal between the infundibulum and isthmus where albumen is deposited around the yolk.

Marsileales: an order of heterosporous, leptosporangiate ferns, including the common water clover fern and *Azolla*.

Mastigophora: a class of protozoans which move by means of flagella.

Meiosis: a cellular process that reduces to one half the number of chromosomes in gamete producing cells. It is reduction division, in which one of each pair of homologous chromosomes passes to each daughter cell.

Meiospore: a spore produced by meiosis, also called a gamete.

Mesentery: double walled membranes which support and protect organs within the coelom of animals.

Meso Form: mid, in the middle, or intermediate.

Micropyle: the opening through the integument of an ovule to the megasporangial wall.

Microsphere: a minute sphere. Also, glass beads approximately 30 μ in diameter.

Mitosis: a process of nuclear and/or cell division which results in the formation of two identical daughter nuclei.

Mitospore: a spore formed from the mitotic process.

Monera: the kingdom of microorganisms classified as neither plant nor animal.

Monoclonal: produced by, being, or composed of cells derived from a single cell.

Monoploid: having or being haploid in a polyploid series of organisms.

Mordant: a chemical that fixes a dye by combining with the dye to form an insoluble compound. Also, a corroding substance used in etching.

Moss: bryophyte organisms in the class *Musci* in the plant sub-kingdom *Embryophyta* which have leaf-like structures bearing sex organs at their tips. Mosses are land plants that do not possess true leaves, stems, or roots.

Moving Phase: in chromatography, the sample dissolved in solvent which travels through the solid or liquid in to which it is introduced.

Mycelium: the hyphal mat, or entire group of hyphae, belonging to a fungal organism.

Mycobacteria: non-motile, aerobic bacteria, such as some saprophytes, and those causing tuberculosis and leprosy, which exhibit growth and physical characteristics similar to fungi. They are difficult to stain.

Myeloma: cancer of the bone marrow.

"Naked Gene": a theory which states that reproductive compounds such as DNA and RNA are able to exist without protective superstructures.

Neuroblastoma: a ganglionic brain tumor.

Ninhydrin: a poisonous crystalline oxidizing agent, $C_9H_6O_4$, used especially as an analytical reagent in chromatography.

Öogamous: a specialized single cell within which one or more eggs are produced.

Öogonium: a specialized single cell within which one or more eggs are produced.

Öospore: a usually thick walled, resting cell, formed by fusion of a sperm with an egg.

Operculum: a little lid.

Ophioglossales: an order of eusporangiate ferns commonly known as adder's tongue ferns. They are the most primitive of modern ferns.

Optical Activity: the ability to rotate the plane of polarized light to the right or left.

Origin: in chromatography, where the sample dots are placed at the inception of the experiment. In anatomy, the point where a muscle connects to a bone which it does not move.

Ovule: a young seed. The megasporangium, plus the enclosing integuments, of a seed plant.

Paper Chromatography: the separation of compounds by a candlewicking procedure which uses paper as the substrate.

Paramylon: a reserve carbohydrate of several protozoans and algae which resembles starch.

Parasite: an organism that obtains its food from another living organism.

Pathogen: a specific, causative agent of disease.

Peat Moss: compacted sphagum, a large genus of mosses that grows in wet, acid areas.

Pellicle Formation: in biology, the thin skin or covering of protozoans such as *Euglena* and *Paramecium*.

Penicillium: a saprophytic blue mold.

Pericardial: that which surrounds the heart. A serous membrane that encloses the heart and the roots of the great blood vessels of vertebrates.

Periderm: an outer layer of tissue, usually protective, on many roots and stems.

Peritoneal: that which surrounds the abdominal cavity. A serous membrane that encloses the abdominal and pelvic viscera.

Permanent Wilting Percentage: the percentage, measured against field capacity, of soil water left after a plant growing in that soil can no longer extract residual water.

pH: the negative logarithm of the effective hydrogen ion concentration in gram equivalents per liter. It is used to express acidity and basicity on a scale from 0 to 14, acid being 0 to 6.9 and basic being 7.1 to 14. pH 7 is neutral.

Phaeophyta: the phylum of brown algae in the plant kingdom.

Phanerophyta: a division of seed-bearing plants consisting of three phyla, the *Cycadophyta*, *Coniferophyta*, and the *Anthophyta*.

Pheromone: a chemical hormone which stimulates behavioral responses.

Photoelectric Cell: a cell whose electrical properties are modified by the action of light.

Photon: a quantum of radiant energy.

Photosynthesis: synthesis of chemical energy storing compounds from radiant energy, especially the formation of carbohydrates in chlorophyll-containing tissues of plants.

Phototaxis: a turning, or positive physical response to light.

Phycomycetaceae: a class of highly variable, lower fungi closely resembling the algae in developmental and structural respects.

Phytophthora: a genus of phycomycetes which causes late blight in potatoes. *Phytophthora infestans* caused the great Irish potato famine of 1845-1846.

Pileus: the cap of a mushroom.

Planck's Law: the fundamental law of the quantum theory, which states that energy transfers associated with radiation such as light or x-rays are made up of definite quanta, or increments of energy proportional to the frequency of the corresponding radiation. This proportionality is expressed by the formula $E = h\nu$, in which E is the value of the quantum in units of energy, ν is the frequency of the radiation, and h is Planck's constant.

Plantae: the plant kingdom.

Plastid: a specialized cytoplasmic body, usually associated with the production and/or storage of food.

Plate-like: in algal identification, a descriptive term used to classify algae whose cells grow in multicellular sheets rather than filamentously or globosely.

Pleural: that which surrounds the lungs. A serious membrane that encloses the lungs in the thoracic cavity of mammals.

Plus and Minus Strains: sexual strains of organisms in the lower *Thallophyta* which will mate together but which have no other discernable differences in form or function.

Polarimeter: an instrument used to determine the quantity of polarization and the angle of rotation of the plane of polarized light.

Polaroid: a trademark name generically used to identify man-made, light weight polarizing devices constructed from plastic.

Pollen: the mass of young male gametophytes of a seed plant, at the stage when they are released from the anther.

Polyclonal: produced by, being, or composed of at least two genetically different cells.

Polyploid: having three or more sets of chromosomes.

Polysomes: also polyribosomes. Clusters of ribosomes linked together by a messenger RNA molecule, forming a site of protein synthesis in prokaryotic cells.

Pour Plate Method: in microbiology, a technique of pouring sterilized agar into a sterile petri dish.

Primitive Streak: formed in fish and bird embryos, a line running axially along the blastodisc. It is the site of inward cell migration during formation of the triploblastic embryo stage, also known as a blastopore.

Prokaryotic: a cellular organism that does not have a distinct nucleus or other membranous organelles, such as bacteria and blue-green algae.

Promycelium: a haploid mycelium formed during the life cycle of rusts and smuts, the fungal orders *Ustaliginales* and *Uredinales*, from which spermatia, or pycniospores, are formed.

Prothallus: the gametophyte of a pteridophyte.

Protista: a kingdom of unicellular organisms.

Protonema: the filament or flat thallus formed by germination of a moss spore.

Proxemics: the study of how humans space themselves within groups.

Psilophyta: a phylum of the oldest known land plants, mostly Paleozoic, in the pteridophyte division of the *Tracheophyta*.

Pteridophyta: a division of the *Tracheophyta* comprising the *Psilophyta*, *Lycopsida*, *Sphenopsida*, and the *Filicophyta*.

Puccinea graminis: a wheat rust.

Pure Culture: in microbiology, a culture of organisms not contaminated by any other living organism.

Pycnia: among rusts, pockets at the surface of the host in which haploid, or monokaryotic spores called pycniospores are borne.

Pyrenoid: a specialized proteinaceous part of a chloroplast, found primarily in algae, upon and around which starch accumulates.

Quantum: a very small increment of energy.

Quantum Theory: a theory in physics which subdivides radiant energy into finite quanta and which is applied to numerous processes involving transference or transformation of energy at the atomic or molecular scale.

Racemic Mixture: a mixture composed of equal parts of dextrorotatory and levorotatory forms of the same compound, which makes the mixture optically inactive.

Reciprocal Centimeter: given a centimeter, *a*, its reciprocal is $1/a$.

Resin Canal: a tubular intercellular space in gymnosperms and some angiosperms that is lined with epithelial cells which secrete resin.

Respiration: any of various energy yielding oxidative reactions in living matter.

Retardation Factor (R*f*): in chromatography, the ratio of the rate of movement of a migrating substance to the rate of flow of fluid wihin the system.

Rhizoid: a structure of root-like form and function, but lacking xylem and phloem.

Rhodophyta: the plant phylum of red algae in the sub-kingdom *Thallophyta*.

Rhizopus nigricans: black bread bold, in the *Mucorales* order of the class *Phycomycetaceae*, in the *Eumycophyta* fungus division of the *Thallophyta*.

Royal Jelly: a pharyngeal proteinaceous secretion of bees used to feed their young, particularly a queen candidate who is fed royal jelly until pupation.

Saccharomyces: yeast, in the class *Ascomycetaceae*, of the *Eumycophyta* fungus division of the *Thallophyta*.

Sarcodina: protozoans which move by pseudopodia, such as amoebae.

Schizomycetaceae: the bacteria (literally, "fission fungi"). Prokaryotic, mostly non-photosynthetic organisms which divide by simple fission.

Sclerotium: a compact mass of hardened mycelium filled with stored food. In some higher fungi, sclerotia become detached from the fungal body under adverse conditions and remain dormant until favorable growing conditions return.

Secretory Cell: epithelial cells which line resin canals and secrete resin.

Sediment: that which settles out of a liquid.

Septate: in botany, having cross walls.

Serial Dilution: liquid which is sequentially diluted by constant increments.

Seta: the stalk which holds the capsule in mosses.

Sexual: relating to the sexes, usually identified as male and female, or plus and minus strains, or gametes, either heterogamous or isogamous.

Short Shoot: the short woody side shoot in gymnosperms and many woody angiosperms which produce leaves and/or reproductive structures.

Sickle Cell Anemia: a chronic inherited anemia in which a large proportion of the red blood cells tend to be shaped like a sickle. These cells do not survive as long as normal cells, nor can they carry as much oxygen. Therefore those with sickle cell anemia suffer from both tissue oxygen deprivation and anemia.

Sieve Cell: the fundamental type of cell in phloem, being long, slender, and thin walled, and having cytoplasm but no nucleus at maturity.

Simple Stain: in microbiology, the staining of a cell or organism with only one stain.

Slant: in microbiology, a test tube partially filled with agar that has been allowed to harden in a slanted position, thereby exposing a maximum agar surface area.

Solid Media: in microbiology, a nutrient broth which is solidified by a material, usually agar, so that the organism may grow on a solid substrate.

Solvent Front: in chromatography, the farthermost area to which the solvent is absorbed or adsorbed.

Sorus: a cluster of spores on the underside of fern leaves.

Spectrophotometer: a photometer, or light meter, for measuring the relative intensities of light in different parts of the electromagnetic spectrum.

Sperm: a male gamete.

Spermatium: a nonmotile cell functioning as a male gamete in some lower plants.

Sphenopsida: the phylum of horsetails, primitive vascular plants in the pteridophyte division of the *Tracheophyta*.

Spontaneous Generation: abiogenesis.

Sporangium: a case or container for spores.

Spore: a mostly one celled reproductive structure other than a gamete or zygote.

Spore Stain: in microbiology, a staining procedure designed to stain the spore walls of spore bearing bacteria one color (usually with carbol fuchsin), and other cellular structures another color, so that the spores are well differentiated.

Sporocarp: modified folded leaves or leaf segments which hold the sporangia of plants, such as in the fern *Marsilea*.

Sporophyll: a leaf which bears one or more sporangia.

Sporozoa: a protozoan which has no means of locomotion. They are exclusively parasitic.

Spring or Summer Wood: wood produced in those seasons. This spurt of growth produces the typical rings of tree trunks which distinguishes wood of this type from winter wood and growth spurts of other years.

Stamen: the male organ of a flower, or, in gymnosperms, the microsporophyll.

Staphylococcus: a genus of nommotile, gram positive, spherical bacteria which tend to cluster together in irregular, grape-like clusters and which include parasites of the skin and mucous membranes.

Stationary Phase: in chromatography, the nonmoving part of the procedure.

Sterigma: a stalk or filament which bears conidia or spermatidia.

Stipe: a short plant stalk. In mycology, the filament or stalk which bears the cap of a mushroom.

Streak Method: inoculum implanted in lines on a solid medium.

Stromata: singular, stroma. A compact mass of fungous hyphae producing perithecia or pycnidia. Also, the colorless proteinaceous matrix of a chloroplast, in which the chlorophyll containing lamellae are embedded.

Substrate: the base on which an organism lives.

Supernatant: the liquid overlying material deposited by settling, precipitation, or centrifugation.

Suspensor: a cell or organ, derived from the zygote but not part of the embryo proper, which in some vascular plants pushes, by its growth, the young embryo deeper into the tissue of the gametophyte.

Taxis: a reflex orientation movement in relation to a source of stimulation, such as heat, light, chemical, or gravity.

Teliosorus: a reproductive organ in some rusts which gives rise to teliospores, which are thick walled chlamydospores. Teliospores constitute the final stage in a rust's life cycle, and after nuclear fusion these spores give rise to the basidia of the plant.

Tendon: a tough cord or band of white fibrous connective tissue which unites muscles with bones, or other body parts, upon which the muscle exerts action.

Tetrameric: adjective for tetrame, which is a molecule, such as an enzyme or polymer, that consists of four structural subunits.

Thallus: a complete plant body which lacks specialized conducting tissues.

Thin Layer Chromatography: a chromatographic process in which the absorbent medium is a thin layer, such as siliceous fibers, usually laid on a non-reactive substance like glass.

Titurate: to mix liquid by pipetting in and out, or back and forth.

Tracheid: the most characteristic type of cell in xylem. It is long, slender, tapered at the ends, with a toughened secondary wall and without living contents at maturity.

Tracheophyta: a sub-kingdom of plants, encompassing all those plants with vascular tissue such as xylem and phloem.

Translocation: the conduction of a soluble material from one place to another in a plant.

Transmittance: the fraction of radiant energy that, having entered an absorbing medium, reaches its farther boundary.

Transmitted Beam (I): those light waves of the spectrum which reach the farther boundary in transmittance.

Triploblastic Embryo: embryonic stage having three primary cell layers.

Turbidity: cloudiness or opaqueness.

Uredosorus: in rust fungi, the reproductive structures which produce uredospores, asexual thin-walled spores which are produced in repeated crops by the uredinial hyphae.

Vent: the external opening of a rectum or cloaca. In birds, reptiles, and amphibians, eggs and feces emerge from the same vent.

Visible Light: light that can be seen by the naked eye.

Vitelline Membrane: a membrane surrounding the yolk in an egg.

Water Holding Capacity: the field capacity, or maximum water content of a soil before it becomes waterlogged.

Yolk Sac Membrane: an extraembryonic membrane developed from an embryo which surrounds the yolk, and which provides nutrients from the yolk to the embryo during development.

Zone Electrophoresis: the separation of fractions into discrete zones, or areas, on a supporting medium by electrophoresis.

Zone of Inhibition: in microbiology, the areas in a lawn of bacteria where bacterial growth does not occur.

Zoospore: a motile, asexual spore.

Zygospore: Zygotes, formed by the fusion of isogametes, which become thick-walled and go into a resting stage.

Zygote: the first cell of a new organism. A cell which results from the fusion of two gametes such as sperm and egg.

Appendix B:

Suppliers of Equipment, Materials, and Teaching Aids

The following partial list contains only those suppliers known to the author. However, since biological equipment, materials, and teaching aids are used heavily by the instructor, and since this usage requires advanced planning and early acquisition, a starting list was thought to be helpful. Listing of any supplier, or the opposite, does not represent endorsement, implied or otherwise. Other suppliers would be included in future editions, if their identities and product lines are brought to the attention of the author.

General Suppliers

Carolina Biological Supply (East Coast)
2700 York Road
Burlington, NC 27215
Toll Free: (800) 334-5551
(in North Carolina, (800) 632-1231)

Carolina Biological Supply (West Coast)
Powell Laboratories Division
Gladstone, OR 97027
Toll Free: (800) 547-1733

Connecticut Valley Biological Supply
P.O. Box 326, 82 Valley Road
Southampton, MA 01073
Toll Free: (800) 628-7748
(in Massachusetts, (800) 282-7757)

Delta Biologicals (East Coast)
P.O. Box 852
Vidalia, LA 71373
Toll Free: (800) 348-9587

Delta Biologicals (West Coast)
P.O. Box 26666
Tucson, AZ 85726
Toll Free: (800) 821-2502

Fisher Scientific
4901 W. LeMoyne Street
Chicago, IL 60651
(312) 378-7770

Frey Scientific
905 Hickory Lane
Mansfield, OH 44905
Toll Free: (800) 225-2629
(in Ohio, collect (419) 589-9905)

McKilligan Supply
435 Main Street
Johnson City, NY 13790
(607) 729-6511

Science Kit and Boreal Laboratories (East Coast)
777 East Park Drive
Tonawanda, NY 14150
Toll Free: (800) 828-7777
(in New York, collect (716) 874-6020)

Science Kit and Boreal Laboratories (West Coast)
P.O. Box 2726
Santa Fe Springs, CA 90670
(West Coast, collect (213) 944-6317)

Triarch
Dept. B — Box 98
Ripon, WI 54971
(414) 748-5125

Ward's Natural Science (East Coast)
5100 West Henrietta Road
P.O. Box 92912
Rochester, NY 14692
Toll Free: (800) 962-2660
(in New York, collect (716) 359-2502)

Ward's Natural Science (West Coast)
11850 East Florence Avenue
Santa Fe Springs, CA 90670
Toll Free: (800) 872-7289
(in Calif., collect (213) 946-2439)

Specialized Suppliers

Animals (rodents), Body Parts, and Chemicals:
Charles River Breeding Laboratories, and,
Charles River Biotechnical Services
251 Ballardvale Street
Wilmington, MA 01887
(617) 658-3333

Cell Cultures, Viruses, and Antisera
American Type Culture Collection
12301 Parklawn Drive
Rockville, MD 20852
Toll Free: (800) 638-6597
(in Maryland, collect (301) 881-2600)

Chemicals
Flinn Scientific
P.O. Box 231
917 West Wilson Street
Batavia, IL 60510
(312) 879-6900

Chemicals
Polysciences
400 Valley Road
Warrington, PA 18976
Toll Free: (800) 523-2575
(in Canada, Analychem Corp.,
Markham, Ontario)

Chemicals
Sigma Chemical
P.O. Box 14508
St. Louis, MO 63178
Toll Free: (800) 325-3010
(Outside U.S. & Canada: collect
(314) 771-5750)

Chromatography and Electrophoresis Equipment and Materials
Pharmacia Fine Chemicals
800 Centennial Avenue
Piscataway, NJ 08854
(201) 457-8000
(also, Pharmacia (Canada), Dorval, Quebec)

Electrophoretic and Micro-Analytical Instruments
Hoefer Scientific Instruments
654 Minnesota Avenue
P.O. Box 77387
San Francisco, CA 94107
Toll Free: (800) 227-4750
(in California, (415) 282-2307)

Electrophoretic and Tissue Culture Instruments
C.B.S. Scientific
P.O. Box 856
Del Mar, CA 92014
(619) 755-4959

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Air Products and Chemicals, Inc. (AIRCO).
Check your telephone directory (or a construction company) to locate a local dealer for this worldwide supplier of specialty and welding gases.

Glassware and Accessories
Bellco Glass
P.O. Box B, 340 Edrudo Road
Vineland, NJ 08360
Toll Free: (800) 257-7043
(in New Jersey, (800) 222-0227)

Radioisotopes
American Nuclear Products
1232 E. Commercial
Springfield, MO 65803
(417) 869-4432

Tissue Culture and Microbiology Products
Gibco Laboratories
3175 Staley Road
Grand Island, NY 14072
Toll Free: (800) 828-6686
(in New York, (800) 462-2555)
(in Canada, Burlington, Ontario
(416) 335-2255)
(in California, Santa Clara, CA
(408) 988-7611)

Audiovisuals & Software

Many universities and government agencies hold extensive collections of films and videos available to the public at reasonable rates of rental. It is advisable to explore local availabilities before looking into the following outlets known to carry biological audiovisuals and software.

Audiovisuals Resources Section
Reference Services Division
National Library of Medicine
8600 Rockville Pike
Bethesda, MD 20209

Beckley-Cardy
114 Gaither Drive
Mt. Laurel, NJ 08054
Toll Free: (800) 257-8338

Bio Learning Systems
Route 106
Jericho, NY 11753
(516) 433-2992

Cambridge Development Laboratory
110 Fifth Avenue, Dept. CT
Waltham, MA 02154
(617) 890-8076

Charles Clark Co.
168 Express Drive South
Brentwood, NY 11717
(516) 231-1220

Educational Images
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Elmira, NY 14905
(607) 732-1090

Eye Gate Media
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Chicago, IL 60618
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Garden City, NJ 11530
Toll Free: (800) 645-8989

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Pleasantville, NY 10570
Toll Free: (800) 431-2050
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Wichita, KA 67201
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St. Petersburg, FL 33709
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150 White Plains Road
Tarrytown, NY 10591

Projected Learning Programs
P.O. Box 2002, Dept. D
Chico, CA 95927
(916) 893-4223

Rex Educational Resources
P.O. Box 2379
Burlington, NC 27216
(919) 229-4800

Schoolmaters Science
745 State Circle
P.O. Box 1941
Ann Arbor, MI 48106
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(in Michigan, (313) 761-5072)

Appendix C

Answer Key

Part One: Instrumentation in the Study of the Cell

1. Discussion: Development of Coacervates

Review: pages 7 - 9

True or false

1. True 2. True 3. False 4. True 5. False

Multiple choice

6. D 7. B 8. A 9. A 10 C

Essay – good answers should include the following:

1. a. Early beliefs in spontaneous generation
   b. Biogenesis
   c. Louis Pasteur
   d. A.1. Oparin and his theory of spontaneous generation

2. a. A.1. Oparin's hypothesis
   b. The steps involved in Oparin's hypothesis
   c. Stanley Miller's experiments

3. a. Coacervate droplets and their physical characteristics
   b. Proteinoid microspheres and their physical characteristics
   c. The characteristics of coacervates and microspheres that resemble living organisms

4. a. Reproduction
   b. Metabolism

5. There are a variety of answers to this question: at the very least, an expansion based on the facts given in the discussion is possible. The student's comments might include the following:
   a. Origin of the earth
   b. Meteors and hydrocarbons
   c. Dust particles found high in our atmosphere
   d. Miller's and Fox's experiments
   e. Energy sources in the universe

2. Discussion: Chromatography

Review: pages 15 - 16

Multiple choice

1. A 2. B 3. D 4. A 5. D

Matching

6. E 7. F 8. A 9. D 10. B

Essay – good answers should include the following:

1. a. ^{14}C tracking of CO_2
   b. Two dimensional paper chromatography
   c. Autoradiography

2. a. Column chromatography
   b. Fractionation of insulin
   c. Amino acid terminal endings

3. Laboratory: Amino Acid Chromatography

Laboratory: pages 19 - 23

1. Answers will vary depending on physical factors and the unknowns chosen by the instructor.

2. Again, answers will vary depending on physical factors.

3. The answers will depend upon the data gathered in #1 and #2. Students should be able to determine the unknowns by correlating the R*f* values with the previously gathered data.

Review: pages 25 - 26

Multiple choice

1. A   2. C   3. A   4. D   5. B

Matching

6. E   7. C   8. D   9. B   10. F

Essay – good answers should include the following:

1. a. Definition of a solvent
   b. Role of solvent in chromatographic processes including such factors as
      1) solubility
      2) timing
      3) R*f*
2. a. Environmental factors such as temperature and humidity
   b. Solubility factors such as amino acid and solvent compatibility
   c. Physical factors such as levelness of the Coplin jar or angle of the thin layer chrpmatography sheet

4. Discussion: Polarimetry

Review: pages 33 - 34

Definitions

1. The ability to rotate a beam of polarized light.
2. A 50 - 50 mixture of enantiomers.
3. Light whose light waves are moving all in the same direction.
4. Enantiomers – molecules which are identical in their chemical properties, as well as being optically active.
5. Molecules which are nonsuperimposable mirror images of each other, such as enantiomers, and diasteriomers, when there is more than one asymetric carbon atom.

Short answer questions

1. See *Figure 4* and discussion on page 4.
2. See *Figures 5* and *6*.

5. Discussion: Spectrophotometry

Review: pages 39 - 40

True or false

1. False   2. False   3. False   4. False   5. True

Essay – good answers should include the following:

1. a. Sir Isaac Newton and the particle model of light and its properties
   b. James Maxwell and the electromagnetic spectrum and its properties
   c. Albert Einstein and photons and their properties
2. See *Figure 2* and discussion on page 5
3. a. Energy bonds of living organisms and their reaction to radiation energies
   b. Types of electromagnetic radiation available in the biosphere

6. Exercise: The Atomic Spectrum of Hydrogen

Exercise: pages 43 - 46

1. When n = 1, E_n in cm^{-1} = -109,737.31. When n = 2, square 2 and divide into -109,737.31, etc. Use the formula found on page 4 of the text.
2. Use the values of question #1 and graph them.

3. Use the formula found on page 7 of the text.
4. Compare the values found in *Figure 4* with those angstrom values listed in *Figure 5*.
5. Compare the values found in *Figure 4* with those in *Figure 3*. Interpolate and fill in *Figure 6*.
6. The greater the E' the greater the value of E', and the smaller the value in angstroms.
7. 7,000Å to 2,500Å.
8. Balmer was limited experimentally to wavelengths in the visible and near ultraviolet regions.

Review: page 47

1. 1.7006×10^{-12} cm^{-1}.
2. 2.5×10^{19} ergs, 5×10^{-4} joules, and 7.195×10^{16} kcal/mole.
3. 14,908 cm^{-1}.
4. 43,487 cm^{-1}, 2,253.672 Å, 8.6974×10^{-19} joules, and 125.16 kcal/mole.

7. Laboratory Enzyme Activity

Laboratory: pages 50 - 70

1. RH and H_2O_2.
2. ROH and H_2O.
3. Students should be familiar with the browning reaction in which fruit goes from fruit color to a brownish discoloration upon exposure to air.
4. The ability to extrapolate intervening line points.
5. So that you are not also measuring solvent absorbence.
6. Readings will vary depending upon laboratory set up and freshness of root.
7. The graph is based on the data in #6.
8. The cuvettes hold 10 ml.
9. and 10. Readings will vary depending upon laboratory set up and freshness of root.
11. Increased concentration of enzyme increases reaction rate up to a certain point.
12. The graph is based on the data in #9 and #10.
13. Use 1.2 ml. H_2O_2 and adjust distilled water accordingly.
14. Readings will vary depending upon laboratory set up and freshness of root.
15. Use 0.3 ml. H_2O_2 and adjust distilled water accordingly.
16. Readings will vary depending upon laboratory set up and freshness of root.
17. Increased substrate increases reaction rate up to a certain point.
18. The graph is based on the data in #14 and #16.
19. Increased substrate and enzyme concentrations increase reaction rate up to a certain point.
20. 21, 22, and 23. Readings will vary depending upon laboratory set up, freshness of root, and exact water bath temperature.
24. This will vary somewhat, depending on the root extract used.
25. The graph is based on the data in #6, #20, #21, #22, and #23.
26. 27, and 28. Readings will vary depending upon laboratory set up and freshness and type of root used for extract.
29. pH will vary depending on local conditions.
30. The enzyme will be most effective at pH just on the acidic side of 7, but this also varies somewhat depending upon the type of root extract used.

31. Consider the most common growing environments available in the biosphere. Plants need to adapt to that which is most commonly available, thus insuring their success.

32. The graph is based on the data in #6, #26, #27, and #28.

33. This will vary somewhat, depending on the root extract used.

34 and 35. If the hydroxylamine does not totally inhibit peroxidase activity, it should certainly slow the reaction down. This depends on the root extract used.

Review: pages 71 - 72

Multiple Choice

1. C 2. A 3. D 4. B 5. A 6. D 7. C 8. A 9. D 10. A

Essay — good answers should include the following:

1. a. pH
   b. enzyme concentration
   c. substrate concentration
   d. temperature

8. Laboratory: Photosynthesis & Bioluminescence

Laboratory: pages 75 - 83

1. Answers will vary depending upon the freshness and variety of spinach used.
2. Data for the graph based on #1.
3. Yes. Students should see two areas of high absorbance in the absorption spectrum of spinach chlorophyll, chlorophyll *a* and chlorophyll *b*.
4. No. Students should see a low point around 550 nm. (green), but photosynthesis continues slightly below 400 nm. and above 700 nm.
5. Round and green.
6. The initial color of both test tubes will be green. The test tube left in the dark stays green, but the test tube exposed to the light changes color, showing that the Hill reaction can only take place in the light.
7. The dye.
8. Water.
9. On the right-hand side of the compound.
10. Yes. No. Light is the catalyst for the reaction, but reaction rate will still be limited by the number of chloroplasts (substrate) available for photosynthesis.
11. Many responses to this question are appropriate.
12. Light.
13. As an energy source.
14. Students should refer to the overall equations listed on pages 73, 78, and 81.

Review: pages 85 - 86

Multiple Choice

1. D 2. C 3. A 4. D 5. C 6. D 7. C 8. C 9. B 10. B

Essay — good answers should include the following:

1. a. Definition of absorption spectra
   b. Definition of action spectra
2. Students should refer to the overall equations listed on pages 73, 75, and 81.

9. Laboratory: Respiration & Fermentation

Laboratory: pages 90 - 92

1. Some of the Durham tubes will be completely full, some will be partially full, and some will be empty.
2. Answer will vary depending upon the physical conditions of the experiment.

3 and 4. The data will depend on the results of #2.

5. The more food the more carbon dioxide production, until toxic levels are reached.
6. There are many problems with this laboratory: not all gas produced is collected and measured; room temperature is not taken into account; molasses is a complex sugar and not subject to easy analysis; etc. However, it does demonstrate qualitative and comparative results.
7. Correctives are numerous but would result in a complex, although quantifiable exercise.
8. This experiment could be run using sucrose as the yeast's energy source. It could be run again using glucose, fructose, amino acids, sulfur dioxide, and vitamins, sequentially, as the yeast's energy sources. Gas production from the different energy sources could then be compared.

Review: page 95

Short answer essay — good answers should include the following:

1. Many responses are appropriate.
2. a. Define fermentation
   b. Discuss the overall equation for fermentation: Sucrose + water $\xrightarrow{\text{yeast}}$ ethanol + carbon dioxide + energy
3. a. Definitions of photosynthesis, glycolysis, and respiration
   b. Overall equations for photosynthesis, glycolysis, and respiration
   c. Comparison of photosynthesis, glycolysis, and respiration

10. Discussion: Electrophoresis

Review: Pages 101 - 102

Definitions

1. The sample to be analyzed is dissolved in a liquid and fractionated in a U tube filled with unstabilized buffer.
2. The sample to be analyzed is dissolved in a liquid and fractionated on stabilizing media which separates the sample into discrete zones, or bands.
3. Biological materials are separated into fractions by zone electrophoresis, and the fractions are allowed to react with immune serum.
4. It is a multicomponent complex of protein and lipids of a characteristic density, molecular weight, size, and chemical composition.
5. It is a molecule's mobility in a solution subject to an electric field.

Essay — good answers should include the following:

1. a. Definitions of electrophoresis and genetic variability
   b. Advantages of studying genetic variability with electrophoresis
   c. Results of electrophoretic studies on genetic variability
2. a. Characteristics of the particle itself
   b. Properties of the electrical field
   c. Nature of the medium
   d. Environmental factors such as temperature and humidity.
3. a. The principle of one gene - one enzyme
   b. The use of electrophoresis to grossly analyze the structure of a protein
   c. The implications of Pauling's results on the genetic concept of dominance and recessiveness.

11. Laboratory: Gel Electrophoresis

Laboratory: pages 108 - 109

1. The LDH proteins will arrange themselves according to the pH gradient.

2. Students should observe the dyed points where LDH activity occurred on the gel. There should be bands of five types of LDH isoenzymes.
3. Observation.
4. Five.
5. Yes. All five dots should contain the same five isoenzymes.
6. Observation. Exact pH will depend on the exact conditions of the experiment.

Review: pages 111 - 112

True or false

1. False - dye
2. False - positive
3. False - isoenzyme
4. True
5. False - ampholytes

Short answer — good answers should include the following:

1. a. In the presence of a lactate, LDH changes NAD to NADH
   b. NADH reduces PMS
   c. Reduced PMS in turn reacts with the dye, tetranitroblue tetrazolium, to form the insoluble colored precipitate, tetranitroblue tetrazolium formazan
2. a. Amphoteric reactions of proteins are based on the amino acid groupings within the protein
   b. Amino acids possess carboxyl and amino groups which become electrically charged when ionized
3. The ability to separate proteins which differ but slightly in their isoelectric points.

12. Laboratory: Cells

Laboratory: pages 116 - 122

1. No.
2. Ribosomes.
3. Yes. It is Brownian movement, not true, purposeful movement. Students may describe it as a shaking or shimmering of life.
4. Yes. Methylene blue stains the cell membrane/wall area darker blue, and the cytoplasm pale blue. The bacteria will be much easier to see.

5 and 6. Observation.

7. Answers will vary depending on the *Euglena* culture and species.
8. Yes.
9. Red. It functions as a light sensitive spot.
10. Observation.
11. Several answers are possible. Students might discuss either the chemical or structural differences between pellicles and cell walls.
12. Positively phototactic.
13. *Euglena* photosynthesize. They also move like animals. *Euglena* can therefore move toward any energy giving light. This is an adaptive advantage.
14. Answers will vary depending on the *Paramecium* culture the class is using.
15. In the food vacuoles.
16. They are changing color to blue.
17. pH.
18. No. However, students may think they see cilia if there is gross movement of substances around the *Paramecium*.
19. Negatively phototactic. Because they are animals not dependent on the sun's energy for food, and it is to their advantage to stay out of the sun's rays to avoid drying out.

20. Probably five, depending on the leaf.

21. Observation.

22. Probably not. If the leaf has a spongy layer of cells, this spongy layer often will have cells lacking in chloroplasts. They would then obtain their energy from the next layer of cells, which do have chloroplasts.

23. Answers will vary.

24. They can see up to five different cell types, by far the most common being the red blood cell. The others will be different types of leucocytes (unless there are blood diseases or other abnormalities).

25. Observation.

26. A nucleus.

27. They do not. They must be manufactured in the bone marrow of humans.

28. Five.

29. Observation.

30. Answers will vary.

31. They are more like each other.

32. No. They are designed for very particular functions with very specific structures. They depend on one another for life support.

Review: pages 123 - 124

Multiple choice

1. A 2. C 3. B 4. C 5. A 6. D 7. A 8. C. 9. A 10. D

Short answer — good answers should include the following:

1. By shape — round, rod, and spiral
2. By movement — flagellate, ciliate, pseudopod, and sporozoid.

Part Two: Diversity of Life and Reproduction

13. Laboratory: Bacterial Staining Procedures

Laboratory: pages 129 - 134

1. Observation.
2. Answers will vary, but students will probably see both rod and cocci in single, double, and other groupings.
3. Answers will vary according to their personal oral bacteria.

4 and 5. Observation.

6. The cells would all appear dark blue to black, with no variation in color.
7. Answers will vary.
8. Blue.
9. Pink to red.
10. Observation.
11. a) The slide and other items are dirty, and the saliva the student is testing was contaminated.

    b) Since the mouth is an aerobic environment, the spore formers would have to be either in the genus *Bacillus* or *Sporosarcina*. Many of them are common soil inhabitants, while others such as *Bacillus anthracis* are the causative agents of disease. Many spore forming organisms of both genera are able to decompose urea.

12. Observation.

13. Because of the waxy lipid substance on their cell walls.

14. Acid alcohol will decolorize almost everything, excepting the cell walls of acid fast bacteria.

15. Hopefully the answer is 'no. Two possibilities for the presence of mycobacteria in the mouth are:

    a) contamination of the slide and apparatus while preparing the student's saliva for examination, and

    b) the presence of mycobacteria in the mouth. The acid fast stain is a diagnostic procedure used in the detection of tuberculosis; there are also many non-pathogenic species which are common inhabitants of soil.

Review: pages 135 - 136

Multiple choice

1. B 2. B 3. A 4. A 5. C

Short answer – good answers should include the following:

6. It will wash off.

7. Answers will vary, but when only one stain is applied, it is a simple stain; when two or more stains are applied sequentially, it is a differential stain, such as gram, spore, or acid fast.

8. They would be spore formers, and the gram stain would vary with the age of the culture.

9. a) So as not to cook and kill the bacteria being inoculated, and

    b) If the petri plate is a disposable plastic dish, heat would warp or melt the plastic.

10. Cocci genera are based on planes of division: if the divisions form doublets, the genus is *Diplococcus*; if the cocci divide in one plane only and form chains of cells, they are called *Streptococcus*; if they divide irregularly, they are *Staphylococcus*; and, if they divide to form cubes of eight, they are *Sarcina*.

14. Laboratory: Isolation of Pure Cultures from Mixtures of Bacteria

Laboratory: pages 140 - 146

1. At first, the bacteria probably will look like background dust to students. They need to practice distinguishing bacteria from debris. Bacterial movement is a shimmering or agitation without purposeful direction. It is called "Brownian movement."

2. True movement is movement in response to stimuli with purposeful direction. Brownian movement is not purposeful and is not in response to stimuli.

3, 4, 5, and 6. Varies depending on unknowns.

7. Gelatin melts above room temperature. In fact, if the room is warm or it is a warm day, students may need to chill the tube before "reading" the gelatine stab.

8 and 9. Varies depending on unknowns.

10. That the bacterium has the ability to reduce nitrates to nitrites for energy.

11. Varies depending on unknowns.

12. That the bacterium has the ability to split off the side chain of tryptophane, $CH_2CHCOOH$, leaving indol for energy production.

13. Varies depending on unknowns.

14. That the bacteria have the ability to use the protein, gelatin, for energy production.

15 and 16. Varies depending on unknowns.

Review: pages 147 - 148

Essay – good answers should include the following:

1. a. A sterilized test tube partially filled with water – tap, distilled, or saline
   b. A sterilized test tube partially filled with a sugar water solution
   c. A sterilized test tube partially filled with a protein solution
   d. A sterilized test tube partially filled with hardened agar
   e. A sterlized test tube partially filled with hardened agar which has been cooled at an angle so that the agar in the test tube has a maximum amount of surface area exposed
2. Inoculate the bacteria in two types of ferments, one maltose and one glucose, the bacteria that grow on the different ferments are the two types. Biochemical activity is one characteristic used for classification.
3. Answers will vary.

15. Laboratory: Isolation of Staphylococcus

Laboratory: pages 151 - 155

1 and 2. Answers will vary.

3. *Staphylococcus* is a gram positive cocci.

4. If colonial growth completely covers a surface area and then foreign substances are introduced, the substance which serves as a growth inhibitor, or antibacterial, can be noted with the naked eye.

5. The most to least effective antibiotic will depend on the type of bacteria under cultivation.

6. The one which tested most effective. There is not much point in prescribing one that is less effective.

7. Findings should vary with the bacterial type under investigation.

Review: page 157

Matching

1. F 2. C 3. G 4. H 5. J 6. I 7. B 8. D 9. A 10. E

Short answer

Because you have selected for growth on blood hemolytic, pathogenic bacteria, the organisms must be prevented from causing contamination.

16. Laboratory: Introduction to the Algae

Laboratory: pages 163 - 174

1. Answers will vary depending on the algae selected.
2. Observation.
3. Students should identify the word *Thallophyta* from thallus (Gr. thallos, a sprout) which means a complete plant body without specialized conducting tissues and without parts resembling roots, stems, and leaves. Those *Thallophyta* which contain chlorophyll pigments are for the most part algae. Other pigments may occur in addition to the chlorophylls. Algae may be single celled plants or multicellular with an organization varying from filaments to plate-like growth or dichotomously branching thalli.
4. Answers will vary depending on the algae selected.
5. See the characteristics listed in the chart, page 163 of the laboratory. Additionally, life cycle characteristics and biochemical activity should be considered.
6. Zoosporangia house zoospores which are produced by mitotic division and hence are vegetative cells. In *Ulothrix*, they are diploid with four flagella. Gametangia house gametes which are produced by meiosis and hence are sex cells. In *Ulothrix* they are biflagellate haploid cells and are isogamous.
7. Observation.
8. They are a product of meiosis.

9. Yes. They are a product of mitosis.

10 and 11. Observation.

12. No.

13. Heterogametes.

14. Diagram.

15. See glossary.

16 and 17. *Spirogyra* is a green alga with reproduction similar to *Ulothrix*. *Oedogonium* and *Vaucheria* are heterogamous like *Fucus*. *Spirogyra*, *Oedogonium*, and *Ulothrix* are green filamentous algae. *Vaucheria* is a yellow-green, coenocytic, filamentous alga. *Fucus* is a brown, flattened, complex, filamentous, and branching alga.

18. Answers will vary depending on what classification system the student is using.

19 and 20. Answers will vary depending on the outside reading sources to which the student has access.

21. Answers will vary depending on the outside reading sources to which the student has access. However, all answers should include the idea that biochemical changes are of a far more fundamental nature than structural and other morphological changes. Color is superficial in that there are many shades and variations possible; however, the photosynthetic pigment itself is not a superficial marker. Pigments may be added, but the primary biochemical method of photosynthesis is a basic indicator in classification.

Review: pages 175 - 176

Multiple choice

1. C 2. B 3. B 4. D 5. A 6. B 7. C 8. C 9. D 10. B

Matching

11. F 12. E 13. I 14. H 15. C 16. G 17. J 18. D 19. B 20. A

17. Laboratory: Introduction to the Fungi

Laboratory: pages 180 - 194

1. Answers will vary.

2. They are algal fungi requiring a complex growth medium which is as yet undetermined.

3 and 4. Chytrids have zoospores and motile isogametes with a single posterior whiplash flagellum, similar to the algae. Most are intracellular parasites, but the apple trap chytrids remain attached to the surface of the host and extract nutrients by means of haustoria, similar to many fungi. Most remain spherical or ellipsoidal, like some algae, and only some develop small coenocytic mycelia, like other fungi. They are the most primitive of the fungi.

Chytrid sporangia release, usually through an operculum, uniflagellate naked swarm cells. These swarm cells function as zoospores or as isogametes, according to the species and the circumstances. There are many chytrids in which only the asexual cycle is known.

5. Answers will vary.

6. Clear.

7. Branched. However, the branching may not be evident if the individual strands are well developed and long.

8. Nonseptate.

9. The hyphae are coenocytic.

10. Yes.

11. Answers will vary. However, most students will say yes.

12. The bread.

13. The sporangia are borne on aerial hyphae called sporangiophores which are supported by strong lateral hyphae called stolons. Mitosis occurs within the sporangium, forming many spores between the outer limiting wall and the central columella.

14. Because they are the product of mitosis.

15. Male and female are terms traditionally used when there is a difference of the meiospores, generally in size. There are no observable size differences in the plus and minus strains of *Rhizopus*.

16. Isogamous.

17. Students should draw a line between all areas where plus and minus strands meet.

18. Mating would have to be initiated between the unknown and known strains of plus and minus fungi.

19. Some fungi can be mistaken for algae, if they possess photosynthetic pigments. Some algae are coenocytic, and many are filamentous, like most of the fungi. The *Phycomycetaceae* possess numerous flagellated spores, similar to the fungi.

20. Saprophyte; also, it can be weakly parasitic on fruit such as strawberries.

21. Lack of propinquity. Most *Rhizopus* in a given area are of the same strain.

22. They are similar.

23. Ascomycete hyphae are multinucleate and septate. Physomycete hyphae are coenocytic.

24. Mitospores are borne in fours at the tips of conidiophores which are divided into two or more branches, each of which terminates in a chain of mitospores. The sexual cycle in most ascosporangia produces meiospores in chains of eight. Often *Penicillium* has been placed in the *Deuteromycetaceae* because the sexual cycle has been so rarely found. In *Penicillium* the ascosporangia hold meiospores in chains of four.

25. Because they are borne on conidia.

26. Answers will vary.

27. Division is not equal.

28. Meiosis occurs in a zygote formed by the fusion of two equal or unequal uninucleate cells. The zygote enlarges to become an ascus, and the diploid nucleus then undergoes meiosis, with the four resulting haploid nuclei maturing into ascospores, or dividing into eight and then maturing.

29. They can be interchangeable.

30. Conidiospores are mitospores borne on conidia. Ascospores are meiospores borne within an ascus.

31. Mitospores are borne on conidia, and eight meiospores are borne within an ascus. Sexually and asexually *Claviceps* is an ascomycete.

32. Answers will vary, but they should include all aspects of agriculture which depend on grain crops.

33. The major portion of fungi life is hidden; the reproductive phase is the only one which "surfaces," a necessity for spore dispersal.

34. For nutrient gathering and support.

35. Observation.

36. On the hymenium the students will see the asci. Each ascus contains eight ascospores in a single row.

37. Because of the angle it is cut at, or because of the maturity of the ascus.

38. The hymenium gives rise to the spores, not only in fungi but in living organisms generally.

39. Eight.

40. Shallow cup shape with a short stalk. Depending on the ascocarp, they can range in size from 2 cm. to as much as 30 - 40 cm. across.

41. Observation and thought.

42. At the base of the stipe.

43. The hymenium bears slender stalks called sterigmata, on which the spores are borne.

44. Observation.

45. Basidiospores are borne on club-shaped structures (basidia) and are entirely the product of a dicaryotic mycelium. Often teliospores (resting stage consisting of two or more cells) are formed. Meiosis occurs in the basidium, with typically four cells produced per basidium. Often the original nucleus of the basidiospore divides mitotically, so that the basidiospore is binucleate.

See the answer to question #28 for a discussion of the sexual cycle in ascomycetes.

46. The *Lycoperdaceae* (puffballs) are very similar to other basidiomycetes, with the exception of the structure of their fruiting bodies and the position of the basidiospores. A puffball has an outer layer, the peridium, with an inner spongy layer of mycelia, called the gleba. Spores are borne on the capillitium which is formed from parts of the gleba. At maturity the peridium tends to rupture, and wind or other disturbances cause the basidiospores to be discharged into the air.

47. Lichens are combinations of algae and fungi. Several different algae and fungi can be involved.

48. Warm and wet.

49. They seldom do much damage to the reservoir host plant, such as the barberry or gooseberry, but they can destroy the other host plant, such as wheat or the white pine.

50. Parasites.

51. In the stem of the wheat.

52. Two, a plus and minus strain.

53. Observation.

54. Yes.

55. Because the cold weather inhibits growth and transmission of the fungus.

56. The barberry is an alternate host of *Puccinea graminis*, but it is not essential. The fungus can grow indefinitely by spreading from one infected grass to the next, especially in areas where the uredospores are not killed by extremely cold winters.

57. New crops of uredospores may sometimes arrive in Canada via wind patterns which sweep the North American continent. These uredospores come from the south where the winters are less severe. The wheat growing regions of Northern Europe do not have this reinfection problem, because they are cut off from their southern neighbors by the Pyrenees, the Alps, and similar mountain masses.

Review: pages 195 - 196

Matching

1. E 2. G 3. F 4. A 5. J 6. B 7. I 8. D 9. C 10. H

Multiple choice

11. A 12. B 13. D 14. B 15. D 16. C 17. A 18. D 19. B 20. A

18. Laboratory: Survey of the Mosses

Laboratory: pages 198 - 205

1. A root-like structure, the rhizoid.
2. Tan.
3. Depends on the species, and the age of the moss plant.
4. It is not a true root because it did not originate as a structure growing in a direction opposite from the stem. It originates out of the stem.
5. Spirals.
6. Two cell layers.
7. Depends on the selection provided by the instructor.
8. It is a rosette at the apex of the moss plant, and it is more than two cell layers thick.

9, 10, and 11. Yes.

12. It depends on the species under study.

13. Observation.

14. They are too large.

15. The number of cells involved. An archegonium is a rather elaborate structure designed to both support and protect the egg and, later on, the zygote and sporophyte.

16. In water.

17. At the lip, down through the neck.

18. They must be removed.

19. The zygote begins life in the archegonium.

20. The archegonium, and then the archegonial, or female gametophyte plant.

21. Water trapped in the junctures between the "leaves" and branches. Of course, mosses cannot live in dry areas.

22. Answers will vary.

23. The capsules are parasitic on the leafy plant.

24. Haploid. They are part of the gametophyte plant.

25. Depends on the species.

26. It is called fertile in that it produces spores, and sterile in that there is no product other than its own growth.

27. There is no need for a capsule if the moss plant's spores are dispersed by water. If dispersal is by wind, or by an elaborate mechanism for "shooting" the spores with the capsule's operculum, then the capsule is a mechanical necessity. The more advanced mosses have capsules.

28. Actually it is extremely difficult to distinguish protonemata from green algae, except by their environment. As soon as the protonemata produce buds, which are the forerunners of the moss plants, the differences are obvious.

29. Gametophyte = haploid. Sporophyte = diploid.

30. Yes. The 1N generations produce gametes, and the 2N generations produce spores.

31. Homothallic.

32. Heterothallic.

33. It is difficult. "Leaves" are two cell layers thick, and originate off branches in a symmetrical spiral.

34. At the apex, or tips of the plant.

35. It is similar.

36. One kind.

37. Two cell layers thick.

38. The students may vary in this answer, but all should include a discussion of the angle at which the "leaves" are attached to the branches.

39. Answers will vary somewhat depending on the specimen under study.

40. By the flattened "leaves" of liverworts.

41. Off the main "branch" or plant body.

42. There is no differentiation of tissue in moss "leaf" structures. They do not arise from a true stem.

43. They are haploid, and multicellular. In fact, in most species they can be seen with the naked eye.

44. On top of the antheridial disk.

45. The student should compare cell shape and cell position, homothally and heterothally. *Polytrichum* antheridia are multicellular but microscopic, while *Marchantia* have an elaborate eight lobed disk structure that is usually about 1/2 cm. thick and quite visible to the naked eye.

46. On the female gametophyte.

47. One.

48. The student should compare cell shape (*Polytrichum* is elongated and *Marchantia* is more flask shaped), and cell position (*Polytrichum* archegonia are located at the apex or plant growing tips, and *Marchantia* bears its archegonia on special upturned branches). The student should mention that one plant is homothallic and the other is heterothallic.

49. Answers will vary.

50. Similarities can be found in form and function. However, the *Marchantia* capsule is photosynthetic.

51. In the spore mother cells.

52. Spores.

53. They grow from the haploid spores produced by the spore mother cells in the diploid capsules.

54. No.

55. Observation.

56. Yes. Algal and fungal groups often do exhibit a definite alternation of generations.

57. Observation.

Review: pages 207 - 208

Multiple choice

1. D 2. B 3. D 4. D 5. A 6. C 7. C 8. C 9. B 10. B

Essay – good answers should include the following:

1. a. *Bryophyta*: multicellular antheridia and archegonia, no specialized conducting tissues, the sporophyte permanently attached to the gametophyte and usually parasitic on it.
   b. *Musci*: gametophyte "leaves" spirally and symmetrically arranged, sporophyte capsule usually with a columella.
   c. *Hepaticae*: gametophyte "leaves" thickened and flattened pads, sporophyte capsule without a columella.
2. a. Life cycles possess a distinct alternation of generations.
   b. The gametophyte plant is photosynthetic, and produces sperm and egg in antheridia and archegonia. The plant is either homothallic or heterothallic.
   c. The sporophyte plant is usually not photosynthetic, and is partially or totally parasitic on the gametophyte. The sporophyte consists of a foot, seta, and capsule. It produces spores mitotically.
   d. The sporophyte plant is well protected from drying out, and at the same time it can disseminate its spores via wind or water, depending on the species.

19. Laboratory: Introduction to the Club Mosses and Horsetails

Laboratory: pages 214 - 218

1. Roots, stems, and leaves. The student may also list reproductive structures, depending on the specimen.

2. To the stem.

3. Relatively small.

4. A single, unbranched mid-vein.

5. Answers will vary depending on the specimen. Usually alternate, but sometimes opposite, or even whorled.

6. Answers will vary depending on the maturity of the specimen.

7. At tips of stems on sporophylls.

8. On sporophylls, often arranged terminally into a cone.

9. Leaf, or vegetative leaf.

10. Roundish, with four sides.

11. Answers will vary depending on the species.

12. Epidermis, cortex, and central cylinder. In the endodermis of the central cylinder are xylem and phloem, and a pericycle. Parenchymatous tissue, some perhaps partially sclerenchymatous, fills the cortex.

13. Roots, stems, and leaves.

14. Roots, stems, and leaves, three cell layers, and all the tissue differentiated in question twelve.

15. A spore.

16. A spore.

17. The gametophyte.

18. So that it can photosynthesize.

19. Organic material in the soil. It is a saprophyte.

20. In the archegonium.

21. A spore is the product of meiosis and comes from a sporangium of the sporophyte plant; a gamete is haploid and comes from the antheridium or archegonium of the gametophyte plant.

22. Those which are aerial and green. Because they are capable of photosynthesis, they are not dependent totally on a phycomycetous fungus for nutrition and can mature much faster than plants incapable of photosynthesis.

23. Answers will vary, because there are many features to consider.

24. Ploidy, attachment, and vascular tissue.

25. Observations and chart.

26. No.

27. Answers will vary.

Review: page 219

Fill in the blanks

1. mother spore cells
2. meiosis
3. archegonia
4. antheridia
5. mitosis
6. gametes
7. gametophytes
8. spores
9. zygote
10. sporophyte
11. sporophyte
12. diploid (2N)
13. parasitic
14. photosynthetic
15. sporophyte

20. Laboratory: Survey of the Ferns

Laboratory: pages 224 - 232

1. Roots, stems, and leaves.

2. At the tips.

3. At the base, or beginning.

4. Near the newer sections of the axis, or underground stem.

5. Rhizome.

6. To the rhizome.

7. Rhizome.

8. They are true leaves, with a complex structure. Most fern leaves, or fronds, are compound (many pinnately), with petioles, blades, and venation.

9. Yes. They primarily grow apically. Evidence will vary depending on the fern being observed, but the existence of fiddleheads, leaves in several stages of growth, and/or reproductive structures might all be cited in the answer to this question.

10. Answers will vary depending on the fern under observation.

11. Observation. Yes, a cambium is present.

12. Answers will vary.

13. Observation.

14. Answers will vary, but an obvious homology is the ability to photosynthesize.

15. Mesophyll composed of palisade tissue, palisade parenchyma, spongy parenchyma, and veins.

16. Venation is reticulate, very similar to venation in flowering plants.

17. Observation.

18. Fern sporophyte is the dominant, large, photosynthetic generation of the plant. The *Lycopodium* and *Polytrichum* are mostly non-photosynthetic, parasitic appendages to the gametophyte generations.

19 and 20. Observation.

21. The embryo develops from the fusion of an egg and a sperm in the archegonium of the gametophyte. The embryo has a foot and a primary root, stem, and leaf. The foot is embedded in the gametophyte and passes the food it absorbs from the gametophyte on to the sporophyte. After the sporophyte becomes an independent plant, the gametophyte dies.

22. Differentiation of tissues and photosynthesis.

23. The leaf emerging from an underwater shortened rhizome, the sporocarp with its spores, and the gametophytes with their archegonia, antheridia, sperms, and eggs.

24. Observation.

25. Big - produces archegonia, little - produces antheridia.

26. Separate gametophytes for male and female.

27. Answers will vary. Both are heterosporous, but one is water living and one land living, and one has tissue differentiation in the leaves and one does not.

28 and 29. Observation.

30. They enjoy a symbiotic relationship. *Anabaena* can fix nitrogen from the air.

31. Answers will vary. However, it might be noted that antheridia mature before archegonia.

32. Two separate male and female gametophyte plants for heterosporous ferns.

33. Answers will vary. However, structures and functions of those structures should be considered in any answer.

34. Answers will vary.

Review: pages 233 - 234

Multiple choice

1. C 2. A 3. D 4. D 5. B 6. B 7. B 8. B 9. D 10. D

Matching

11. C 12. D 13. A 14. E 15. B

21. Laboratory: Introduction to the Gymnosperms

Laboratory: pages 236 - 252

1. By describing roots, stems, leaves, perhaps reproductive structures, if they are on the specimen plant provided by the instructor.

2. Again depending on the specimen provided for observation, the student should note the unusually long stem which develops before the first leaf. Growth is apically meristematic, and many cycads have short, subterranean stems. The age of a plant can be computed from the number of leaf bases, the number of leaves in a crown, and years' duration of a crown.

3. Many have short, underground stems, like the rhizomes of ferns. Leaf arrangement and structure are similar, although cycads have a stiffer structure. Reproduction in cycads is very similar to the extinct seed bearing ferns. Many ferns are hereosporous, like the cycads.

4. The leaves have a heavy, thick cuticle with a thick-walled epidermis. The stomates are basically confined to the lower surface and are usually sunken in small pockets. These adaptations all enable plants to withstand water loss in a dry climate.

5. Xylem and phloem arrangements, and scanty secondary thickening of these vessels, are similar in ferns and cycads. Fossil cycads also have branched microsporophylls like ferns, but students will have to be told this unless prepared slides of the *Bennettitales* or *Caytoniales* are available.

6. Seeds, a strongly defined taproot, the heavy cuticle on the leaves, and the aggregation of sporophylls into a cone.

7. Apically.

8. Staminate strobili and sporophylls are longer and more slender than ovulate ones. Pollen develops from a microspore mother cell by meiosis to form a tetrad of microspores. The megaspore mother cell produces by meiosis one large megaspore.

9. Because they are multicellular and enclosed in two cell layers.

10. A pollen grain is composed of three cells, the tube cell, a generative cell, and the prothallial cell. It is surrounded by two cell layers from the original wall of the microspore called the exine and the intine.

11. Many — the exact number depends on the specimen.

12. It avoids being dried out when subjected to unfavorable weather conditions.

13. By wind, water, or insect.

14. Pollination.

15. Pollination in gymnosperms is the transfer of pollen from the microsporangium to the micropyle of the ovule. Fertilization is the fusion of sperm and egg, after the growth of the pollen tube has been successful.

16. Answers will vary.

17. One.

18. Parasitic.

19. Answers will vary depending on the specimen.

20. They protect the ovule.

21. Integument.

22. No.

23. To the pollination drop at the micropyle.

24. The cytoplasm of the pollen tube.

25. Seed coat, and an embryo with a primary root, shoot, and two cotyledons.

26. Answers will vary. Primarily, however, the megasporangium, megaspore, and female gametophyte are all dependent upon the sporophyte for sustenance. The embryo sporophyte has a food reserve built into the seed, compliments of the sporophyte.

27. Fertilization without the necessity of a water environment.

28. Answers will vary.

29. Both are dioecious, both have pollen tubes which develop similarly (like a fungal haustorium), and both have sperms with a spiral band of flagella at one end.

30. Shape, size, cell wall thickness, and cell contents.

31. Epiderm, periderm, cambium.

32. The bark.

33. Yes.

34. Yes, more or less.

35. Yes.

36. Xylem.

37. Answers will vary.

38. Yes.

39. Yes. Sieve cells.

40. The xylem and phloem are initiated from the cambium.

41. Yes.

42. This will depend on the specimen.

43. Xylem.

44. Living cells versus dead ones.

45. The tissues of the bark.

46, 47, and 48. Observation.

49. Answers will vary, but they should all include the comment "long."

50. Pitted.

51. The xylem.

52. Observation.

53. Xylem, ray parenchyma and tracheids, and resin ducts.

54. Shape and content.

55. Answers will vary but phloem cells should be one cell type mentioned.

56. If you saw xylem cells.

57 and 58. Observation.

59. The short shoots which attach the cone to a branch.

60. Modified leaves, or microsporophylls, with microsporangia.

61. Answers will vary from two to fifteen, depending upon the species under observation.

62 and 63. They are similar.

64. Most have three cells, again depending on the species.

65. Answers will vary, but should include a statement as to structure.

66. Yes. Structure.

67. Three, again dependent on the species.

68. Because of differences in both structure and function.

69. Pollen grain, developing microspore.

70. Upon maturity.

71. Answers will vary depending upon the species.

72. By wind.

73. On the pollination drops exuded by the micropyles.

74. To the micropyle.

75. An egg.

76. Answers will vary depending upon the species. Some cones release seeds almost immediately and fall off. Others remain attached for a season or for several years, protecting the seeds, and growing themselves. Some only open after the trauma of fire. Others open after a set period of time before seed release.

77. Answers will vary, but the terms parasitic and protective should be mentioned.

78. The embryo.

79. The gametophyte formed it. The sporangium is modified to hold the new sporophyte and protect it. The integument protects it, and the parent sporophyte made it.

80. On the dorsal surface of the modified sporophylls.

81. One.

82. Observation.

83. The seed has a seed coat, or hardened integument.

84. A stamen is a microsporophyll.

85. An ovule is a young seed, with a protective integument. A sporangium is simply a container for spores.

86. An integument is the forerunner of a hardened seed coat, a sporangium is not.

87. The radicle and the hypocotyl.

88. The tips of the cotyledons usually remain enclosed with the seed coat for some time before it is pushed off. Also, the first true leaves are borne separately on the main shoot axis.

89. It is absorbed into the new sporophyte.

90. Depending upon student stress, many answers are possible.

Summary: small (measured in inches), more than 300 feet tall, excurrent branching, minute, 15 feet long, pinnately compound to needle-shaped, deciduous, short shoots, 2, 5, staminate, ovulate, apical, cones, dorsal, pollen, sporophylls, short shoots, ovules, one, one, one or two, embryo, archegonium, micropylar, generative cell, the pollen tube, zygote, embryo, radicle, hypocotyl, and epicotyls, ovule, seed, the new sporophyte, pollination, fertilization.

Review: pages 255 - 256

Matching

1. C 2. E 3. A 4. B 5. D

Multiple choice

6. D 7. C 8. A 9. A 10. B

Essay — good answers should include the following:

1. a. A cone is a reproductive structure made of modified leaves called cone scales or sporophylls.
   b. There are staminate and ovulate cones.
   c. If ovulate, they contain megasporangia with integuments which form ovules and later seeds. If staminate, they contain microsporangia which give rise to microgametes or pollen grains.
2. a. The pine tree, being a conifer, produces staminate and ovulate cones on the same plant. Each cone bears two sporangia on its surface.
   b. In each ovule, the megasporangia contains one megaspore mother cell which divides by meiosis to form four haploid megaspores, three of which disintegrate, leaving one which divides mitotically to form a multicellular haploid megagametophyte. The megagametophyte will form two to five archegonia each containing a single large egg.
   c. In each microsporangium are many microspore mother cells, each of which undergoes meiosis to form four haploid microspores, which divide mitotically to form three-celled pollen grains.
   d. At maturity, the microsporangia burst open, scattering the pollen, which are transported by wind to the pollination drop at the micropyles of the ovules. The integument swells and closes around the micropyle, trapping the pollen. The pollen tube grows, fertilization occurs, and a zygote results. The seed is born.

Part Three: Animal Growth and Behavior

22. Discussion: Hybridomas and Monoclonal Antibodies

Review: pages 265 - 267

Multiple choice

1. C 2. B 3. A 4. D 5. A 6. D 7. A 8. D 9. C 10. B

Short answer

11. As each *B* cell matures in the bone marrow, it becomes committed to the synthesis of antibodies which recognize a specific antigen, or molecular pattern. The descendants of each such cell retain the same specificity, and thus they form a clone of immunologically identical cells.
12. Cancers fundamentally represent failures of the immune system, in that the system does not detect and destroy deviant cells as they usually do. Normal cells are transformed into cancer cells in many ways, with several categories of triggering agent. Both the triggering agent and the resulting cancer cell have bypassed the immune defense system.
13. Self relates to those molecules, cells, and tissues which belong uniquely to an individual. Nonself relates to those molecules, cells, and tissues which are alien to the individual.
14. Both *B* and *T* lymphocytes are produced in bone marrow. *T* lymphocytes are further differentiated in the thymus. *B* lymphocytes manufacture antibodies. *T* lymphocytes variously attack invading intracellular organisms and control the overall immunological response of an individual.
15. Hybridomas are hybrid cells of a cancerous tumor and an antibody producing lymphocyte. Hybridomas, resulting from the fusion of one lymphocyte cell and one tumor cell, secrete only one type of antibody, or a monoclonal antibody.

23. Laboratory: Creating a Heterokaryon

Laboratory: pages 272 - 280

1. NIE 115 cells are not malignant. They should be only one cell layer deep and attached to available surface areas.
2. Answers will vary.
3. Observation.
4. NS 1 cells are malignant bone marrow cells and should show no observable organized orientation. They may clump together and do not necessarily have to be attached to available surface areas.
5. Answers will vary.
6. Observation.
7. See answers one and four for colonial morphology. In terms of cellular structure, NIE 115 cells are secretory and have a large cytoplasmic area in proportion to the nucleus. Students may be able to see what looks like droplets attached to the cell membrane. NS 1 cells are non-secretory and their cytoplasmic area is smaller in proportion to the nucleus. There should be nothing attached to the cell membrane. Students may also note an overall difference in nuclear size between the two cell types.
8. These are mammalian cells (mouse) and should be kept at body temperature.

9 and 11. Hopefully no, unless they have grown more confluent.

10 and 12. Nothing should have yet occurred which would contaminate or otherwise disturb the cells.

13. Hopefully, yes. If not, cultures have been confused.
14. Observation.
15. NIE 115 cells are not malignant and should be only one cell layer deep, attaching themselves to available surface areas. Students should have the same answer here as they had for question #1.
16. Observation.
17. NS 1 cells are malignant bone marrow cells and should have no observable organized orientation. They may clump together, and they do not necessarily have to be attached to available surface areas. Students should have the same answer here as they had for question #4.
18. They should be the same since they are studying the same cells.
19. Hopefully, yes. Not every laboratory group will necessarily achieve fusion, because of carelessness somewhere in their procedures. At least half of the groups should have been successful.
20. Answers will vary. A successful lab group should be able to find six to twelve such cells.
21. Answers will vary. Students probably will be able to respond that there are three to six such fused cells.
22. Observation.

23. The NIE 115 cell line.

24. The NS 1 cell line.

25. Answers will vary, but they may include any or all of the traits listed on the first and second pages of this laboratory.

Review: pages 283 - 284

Matching

1. G 2. H 3. A 4. B 5. J 6. I 7. F 8. L 9. C 10. E

Essay – good answers should include the following:

1. DMEM = Dulbecco's modified Eagle medium, and SF DMEM = serum free DMEM, are culture media. PEG = polyethylene glycol, causes cell membranes to lose their individual identity markers, making fusion possible. PBS = phosphate buffered solution, provides a media free solution in which to wash cells free of other solutions.

2. Thought question for which answers may vary widely.

24. Laboratory: Chicken Embryology

Laboratory: pages 289 - 299

1. Identification.

2 and 3. Observation.

4. Outside the body, on the right side. The heart should be beating.

5, 6, and 7. Will vary.

8. Yes.

9, 10, and 11. Observation.

12. Will vary, however, the heartbeats per minute should be slower than the two-day-old chick heartbeats.

13. Answers will vary.

14. Blood is pumped from the heart through the aortic arches in the region of the pharnyx to the vascularized yolk sac membrane.

15. The amniotic sac with the inner amniotic and outer chorionic membranes.

16. Removal of wastes and respiration.

17. Observation.

18 and 19. Answers will vary.

20 and 21. Answers will vary. However, the older the embryo the slower the heartbeat rate should be. In general, the larger the body volume, the longer it takes for blood to circulate, and the more time elapses between heartbeats. Also, the more active the organism, the higher the metabolism and the faster the heartbeat in proportion to body size.

22, 23, and 24. Answers will vary.

25. It should have shrunk considerably in size, because the embryo has used it as a food source.

26 and 27. Head size in proportion to a chicken's trunk will become smaller as the chicken matures. Similarly, eye size in proportion to a chicken's head will become smaller as the chicken matures.

28 and 29. No. Those body structures needed first for survival tend to develop first. Hence, legs develop before wings in all birds.

30. Observation.

Review: pages 301 - 302

Multiple choice

1. B 2. G 3. H 4. A 5. C 6. E 7. D 8. F 9. B 10. C

11. B 12. A 13. B 14. B 15. B 16. C 17. A 18. B 19. D 20. C

25. Laboratory: Cat Dissection

Laboratory: pages 304 - 344

1. Answers will vary.

2. Eight.

3. Answers will vary.

4. Seven on front feet, and five on hind feet.

5. On their toes. Called technically digitigrade.

6. Soles, pads, and foot position.

7, 8, and 9. Observation.

10. Answers will vary. Juvenile cats do not have molar teeth.

11 and 12. Chart.

13 thru 21. Observation.

22. Answers will vary.

23. The type of cells within the stomach tells you what part of the stomach it is from. The cardiac and pylorus both have deep-branching mucus glands. The fundus has deep gastric pits lined with cuboidal cells.

24. The fundus.

25. Observation.

26. Answers will vary.

27. Observation.

28. Caudally.

29. Observation.

30. External respiratory openings, located on the face.

31. The cavity above the soft palate in the mouth where the nares join the pharynx, a common opening for digestion and respiration.

32. The part of the vertebrate alimentary canal between the mouth and the esophagus. Also a part of the respiratory tract between the nasopharynx and the trachea.

33. The flap of tissue above the larynx. Also, the elongated space between vocal cords in the larynx.

34. Voicebox.

35. Windpipe.

36. Cartilage.

37 and 38. Observation.

39. Yes.

40. Observation.

41. Yes.

42 and 43. Chart.

44 thru 48. Observation.

49. Answers will vary.

50 and 51. Chart.

52 and 53. Observation.

Review: pages 347 - 348

Multiple choice

1. C 2. D 3. A 4. A 5. B 6. C 7. C 8. D 9. B 10. A

11. A 12. D 13. B 14. A 15. A 16. B 17. B 18. A 19. D 20. B

Matching

21. C 22. G 23. F 24. F 25. B 26. G 27. A 28. E 29. D 30. G

26. Laboratory: Animal Behavior

Laboratory: pages 353 - 371

1 and 2. Answers will vary, but since fruit flies generally avoid light, the fewest number should be closest to the light.

3. Observation.

4. Negative phototaxis.

5. To avoid having the experiment influenced by geotropism.

6 and 7. Answers will vary, but more flies should be in the top section than in the bottom.

8. Observation.

9. Negative geotaxis.

10. To ascertain that no factors other than gravity influenced fly behavior.

11. Phototaxic response is greater than geotaxic response in fruit flies.

12. Answers will vary.

13, 14, 15, and 16. Answers will vary, but more flies should be in the food section and the least in the original vial section.

17. Observation.

18. Positive chemotaxis.

19. Negative chemotaxis.

20 and 21. Answers will vary.

22 and 23. Observation.

24 and 25. Answers will vary.

26, 27, 28, 29, and 30. Observation.

31. The one which entered the tank first because he may have established territoriality.

32. Observation.

33. Answers will vary, but the fish first into the tank had the opportunity to establish territoriality.

34. Agonistic behavior. Students should specify.

35. Observation.

36. Less. Models mimic certain fish behaviors but not all.

37. Observation.

38. No (unless the colony was otherwise disturbed). The drone has no importance in the routine functioning of the hive.

39. Observation.

40, 41, and 42. Answers will vary.

Review: pages 373 - 374

Multiple choice

1. D 2. D 3. E 4. C 5. D 6. A 7. E 8. D 9. C 10. A

Essay

1. Answers will vary. If students choose to discuss communication patterns in bees, they must cite both the waggle and round dance as communication forms.
2. Taxic: a reflex action or orientational reaction in response to a physical stimulus. Types of stimuli to elicit taxic responses are heat, light, gravity, and chemicals.
3. Conflict behavior, or agonistic behavior, involves threat displays which are attempts to frighten territorial invaders short of physical conflict. Threat displays are of benefit to an organism because they avoid the possibility of injury or death. Conflict behavior is related to courtship and reproduction because animals defend their reproductive potential by protecting nest, mate, and young.

27. Laboratory: Human Spacing Behavior

Laboratory: pages 378 - 399

All questions, numbers 1 through 53, involve responses based on individual observations of specific situations. Answers to all questions will vary greatly.

Review: pages 401 - 402

All questions, numbers 1 through 3, involve responses based on individual observation to specific situations. Answers to all three questions will vary greatly, depending upon the type and quality of classroom discussion and laboratory observation the student has achieved.

Part Four: Plant Growth Relationships

28. Laboratory: Plant Growth

Laboratory: pages 409 - 432

1 and 2. Observation.

3. Student answers will vary somewhat, but they should list three groups, those which stimulate growth, like IAA, those which have no effect, like water, sucrose, and maleate, and those which inhibit IAA, like CaCl.
4. So that the coleoptile cells cannot be influenced by chemicals traveling from other parts of the plant.
5. Answers will vary.
6. Answers will vary depending upon markings originally placed upon the plant.

7 and 8. Observation.

9. Everywhere.
10. Areas toward the tip of the stem.

11 and 12. Observation.

13. Everywhere.
14. This answer will depend somewhat on the age of the leaf when it was first marked. If it was a very young leaf, longitudinal growth will be marked. If it was a juvenile leaf, growth will be most marked near the midvein in a direction at right angles to the petiole.

15 and 16. Observation.

17. Mostly at the root tip.
18. The tip.
19. Observation.

20. Corn seedlings, like most plants, are positively phototropic, and the phototropic response primarily occurs in the growth tip. Therefore, the growth tips in groups #1 and #5 should have bent toward the light, while groups #2 and #4 should not have been affected by the light, and group #3 should have exhibited a limited positive response to the light.

21. Observation.

22, 23, 24, and 25. Dish #4 should have shown the highest percentage of lettuce germination among those dishes in which light or its absence was the only factor. Dish #9 should also have shown a similar percentage; even though it was exposed to sunlight, the kinetin should have removed the inhibitory effects of the far-red light (irradiation) waves in sunlight. Lettuce seed germination is stimulated by red light and reversibly inhibited by far-red light. IAA, added to dish #7, should have had no effect on this process. However, GA does promote seed germination and the breaking of dormancy, and the student may have had some germination in dish #6. These results taken as a whole are evidence for the existence of a pigment system which absorbs light wavelengths.

U.S. government scientists at Beltsville, Maryland have conducted a series of investigations in this area and have concluded that this pigment system is based on phytochromes, pigmented proteins. Temperature interacts with light in the germination of lettuce seed, and this variable was not taken into account in this experiment. Lettuce seed germination promoted by red light may be thermally reversed as well as being reversed by far-red light. Also, there is a decrease in lettuce seed sensitivity to light with temperatures above 25° C. What were the temperatures under which the experiment was conducted?

26. Observation.

27. Peas demonstrate light responses similar to those of lettuce. See the above discussion, questions #22 through #25.

28 and 31. Observation.

29 and 32. See question #27.

30 and 33. GA generally promotes the growth of intact plants, and promotes bolting and flowering in long-day plants.

34. Observation.

35, 36, and 37. Pot #1 is the control group from which the other three pots may be measured. Pot #1 should demonstrate that with removal of the apical bud, lateral buds will grow. Lanolin does not change this reaction. Pot #2, treated with 1% IAA in lanolin, demonstrates continued suppression of the lateral buds, showing that IAA is the auxin in the apical bud which suppresses lateral bud growth. The nutritional status of the plant can influence this suppression. Therefore, if this experiment were to be conducted in different soil types, the results would vary. Pot #3, treated with 1% GA in lanolin, demonstrates that GA does not inhibit the formation of lateral buds, but the student might note that GA promotes internode elongation which IAA suppresses. Pot #4, treated with both IAA and GA, demonstrates that GA synergistically increases the action of IAA on lateral bud growth.

38. Observation.

39. Morning glories belong to a group of plants in which the photoperiodic response may be modified or even reversed by changes in temperature. Consequently, answers to question #39 will vary depending upon the temperature and its variations during the experiment, and for each class the results of this experiment will depend upon the temperature, day and night, in the environmental chamber. Morning glories behave as a long-day plant at night temperatures of 13° C., as a short-day plant at a night temperature of 20° C., and as a day-neutral plant at a night temperature of 18° C. This complex relationship between the effects of temperature and the photoperiod points up the fact that environmental factors governing plant growth act by favoring certain chemical reactions in the protoplasm, at the expense of others. The behavior of the plant is determined by the chemical reactions which occur under a given set of environmental conditions.

40. Answers will vary.

41. Observation.

42. Florigen rate seems to be affected not only by day-night duration but also by temperature. See question #39. Consequently the rate of florigen varies in relation to photoperiod and temperature interactions. Florigen is so potent that experiments have been conducted in which a short-day and a long-day plant, grown under short-day conditions, were grafted together, and the florigen from the short-day plant forced blooming in the long-day plant under short-day conditions. The reverse also is true. Experiments such as this suggest that all florigen compounds are the same, or very similar, and that florigen is not species specific. Isolation and description of the chemical would constitute a scientific breakthrough.

43. Answers will vary, but all students should understand that the potential of having florigen available to the horticultural industry would be immense.

Review: pages 433 - 434

Essay – good answers should contain the following:

1. Auxins do not affect lettuce seed germination, and gibberellins and cytokinens promote germination. How? Lettuce seed is stimulated by red light and inhibited by far-red, or infrared. Cytokinens stimulate lettuce seed in the same manner as red light, but the reaction is not inhibited by far-red radiation. It is probable that cytokinens promote seed germination in concert with IAA. Gibberellins are natural growth hormones.
2. Short-day plants are those in which flowering is initiated as soon as the day length falls below a critical photoperiod of about 12 to 14 hours. Short-day plants usually bloom in the fall or spring. Long-day plants are those in which flowering is initiated only when the day length is more than a critical period of 12 to 14 hours. Under artificial conditions, long-day plants will flower when continuously illuminated. Long-day plants usually bloom in the summer. Day-neutral plants, such as dandelions and tomatoes, bloom under a wide variety of conditions. Short-day plants might better be called long-night plants, and vice versa, because it is actually the length of the night, rather than the day, which impacts the flowering process. Interruptions of only a few minutes in the length of the night may interfere. Street lights or even car lights have been known to disrupt flowering.

   See questions #39 and #42 of this laboratory for an answer as to how florigen interacts with gibberellin in the flowering response, and how this relationship interacts further with day/night length.
3. Phototropism, a response to light, is regulated by IAA in the growth tip of a plant. Apical dominance is also maintained by the presence of IAA in the plant's growth tip. Kinetin, in the presence of IAA, stimulates lateral bud growth. Apical dominance is controlled by a balance of concentrations between endogenous kinetin-like substances and IAA. Gibberellin promotes growth, especially internodal growth, bolting, and flowering. It has no effect on apical dominance by itself, but its effects with IAA are additive.

29. Laboratory: Plant and Water Relationships

Laboratory: pages 438 - 449

1, 2, 3, and 4. Observation.

5 and 6. Answers will vary.

7, 8, 9, 10, 11, 12, 13, 14, 15, and 16. Observation.

17. Yes. Total imbibition will be similar, but the initial rate of imbibition will increase as temperature increases, up to 25° C.

18, 19, 20, 21, and 22. Observation.

23, 24, 25, and 26. Answers will depend on the plant available.

27, 28, and 29. Observation.

30. Answers will vary. Factors such as corn seed age and variety, and the temperature at which the pot was stored, also influence germination. These variables were not factored into this experiment. The results of the experiment will be valid for the seed variety and growing conditions.

31. Observation.

32. Xylem absorbs water in the root system and transports it upward into the plant, and phloem transports the water, nutrients, and minerals dissolved in water to all parts of the plant. If the xylem were blocked, the plant could not absorb water. The plant would lose its initial water content through transpiration to the atmosphere and be unable to absorb more water from the flask. If the phloem were blocked, the plant would continue absorbing and using water from the flask. Therefore, the set-up with blocked phloem will show a greater weight loss. Water is taken up by xylem, distributed by phloem from the xylem, and eventually lost through plant transpiration, hence the correspondingly greater weight loss of the blocked phloem set-up.

Review: pages 451- 452

Short answer

1. Water holding capacity is the field capacity, or maximum water content of a soil without waterlogging occurring.
2. Permanent wilting percentage is the percentage of soil water left after a plant growing in the soil can no longer extract any of the residual water.

3. Xylem absorbs water from the root system and carries it upward for use in the plant. Phloem transports food and minerals in a water solution downward and to all parts of the plant.

4. Temperature increases plant imbibition, up to 25° C.

5. Adequate soil water is essential for seed germination. The process of germination may be defined as that sequence of steps beginning with the uptake of water and leading to the rupture of the seed coat by the radicle or the shoot. Cell divisions and enlargements in the embryo and an overall increase in metabolic activity accompany these steps, all of which require water.

30. Laboratory: Mineral Absorption, Use, and Translocation in Plants

Laboratory: pages 458 - 469

1, 2, and 3. Observation.

4. No. Fe^{59} should have been absorbed into the barley roots in two stages. The initial stage should have been a rapid, passive uptake, followed by a period of equilibrium, and then another stage in which uptake was more slowly but uniformly accomplished. The second stage is demonstrative of an active, metabolic mineral uptake by a plant.

5. Theoretically, if iron uptake by a plant is passive, temperature should have no effect. If iron uptake is active, uptake should increase as temperature rises. Because the second stage of iron absorption is metabolic, the student should have noted that temperature increase does indeed increase Fe^{59} uptake.

6. The amount of a similar competing ion available in solution is inversely proportional to another mineral's absorption. This is termed the carrier theory of absorption, and it is equivalent to saying that the plant cell's active carrier sites determine the proportion of iron/manganese taken up. The proportion of iron or manganese in solution affects the uptake of the other.

7. See questions #4, #5, and #6. Those conditions which favor optimum plant growth favor the most active iron absorption rate. The absence of competing ions also favors the increased absorption of Fe^{59}.

8 and 9. Observation.

10. Answers will vary, but students should note how pH affects absorption rate. Mineral uptake proceeds best under the optimum growing conditions for the plant, and this includes pH. If a solution initially was not within the best pH perimeter, the plant would not grow well in that solution. If a solution initially was within the plant's pH perimeter, but the pH of the solution changed because of utilization by the plant, plant growth would lessen over time.

11. Observation.

12. The complete solution.

13 and 14. Answers will vary, because all missing nutrients are vital to growth. Students will find that carbon must be applied for growth, and depending on location, might find that sulfur is the least vital for growth. However, sulfur is a major component of air pollution, and the plant may receive its sulfur requirements from the air.

15. Iron functions in the synthesis of chlorophyll and also as a constituent of several oxidative enzymes. Studies have found that when iron is absorbed in the dark, uptake of iron is favored by the root over the shoot. This is the opposite of what happens in sunlight. Consequently, the plant grown for four weeks in a radioactive iron solution should have a strong concentration of radioactive iron in the roots and stems. The plant grown for two weeks in radioactive iron, and then for two weeks in a non-radioactive solution, will have definite but weaker radioactivity more evenly distributed throughout the plant.

Review: pages 473 - 474

Essay — good answers should include the following:

1. a. Translocation: the conduction of a soluble material from one place in a plant to another.
   b. It occurs in order to distribute and re-distribute minerals and nutrients to the areas of greatest need.

2. See pages 453 - 454 where fully discussed.

3. a. Radioactive tracers allow scientists to follow the translocation of salts from one area of a plant to another.
   b. Radioactive tracers allow scientists to quantify the amounts of mineral salts in different areas of a plant, which provides clues as to how, how much, and where a plant uses the mineral.
   c. Radioactive tracers, by their redistribution in plants, chemically signal how certain plant life stages are initiated or finalized, and at what points these stages or developments become irreversible.